

AN INTEGRATIVE STUDY OF THE SAILFIN MOLLY, *POECILIA LATIPINNA*
(ATHERINOMORPHA: POECILIIDAE) RESPONSE TO HYPOXIA.

By

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AN INTEGRATIVE STUDY OF THE SAILFIN MOLLY, *POECILIA LATIPINNA*
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In this study, I quantified mechanisms compensating for hypoxia (oxygen scarcity) in the sailfin molly, *Poecilia latipinna*, focusing on interactions between behavioral and physiological responses, fitness consequences, and implications for interdemc variation. I focused primarily on *P. latipinna* collected from a salt marsh at Cedar Key, Florida, where dissolved oxygen concentration is highly variable, both spatially and temporally, and hypoxia ($<2.0 \text{ mg l}^{-1} \text{ O}_2$) is common (occurred on 85% of sampling dates). Short-term behavioral and physiological responses appear to permit *P. latipinna* the flexibility to deal with extreme hypoxia. Fish acclimated to chronic extreme hypoxia (six wk at $1.0 \text{ mg l}^{-1} \text{ O}_2$) exhibited higher hemoglobin [Hb] and red blood cell concentrations (RBC), and a 15- 20% lower critical oxygen tension (P_c) than fish acclimated to normoxia. The use of aquatic surface respiration (surface skimming behavior, ASR) was an immediate

response to hypoxia that decreased over the acclimation period suggesting that physiological compensation reduces the threshold for ASR. Reproduction continued under extreme hypoxia, and there was no effect on survivorship. Only males acclimated to hypoxia showed a lower rate of reproductive activity and lower condition than normoxia-acclimated males. However, the oxygen requirements of female mollies (measured as routine metabolic rate) increased with development of their gestating brood, as did time allocated to ASR under hypoxic stress, which may affect vulnerability to aerial predators.

The scale of the analysis was increased by quantifying variation in hypoxia compensation between *P. latipinna* from a periodically hypoxic environment (Cedar Key salt marsh) and *P. latipinna* from the well-oxygenated waters of the Santa Fe River. *Poecilia latipinna* from Cedar Key spent less time conducting ASR, had lower gill ventilation rates, and spent less time near the surface than conspecifics from the Santa Fe. Hypoxia acclimation reduced the critical oxygen tension in both populations, but population-level differences were maintained. Cedar Key fish had 14% greater gill surface area relative to their size than Santa Fe River fish, perhaps in part accounting for their greater tolerance to hypoxia. The source of these interdemographic differences remains to be determined and will be important to our understanding of the role of hypoxic waters in the maintenance and promotion of physiological diversity. I conclude that plastic behavioral and physiological responses in *P. latipinna* compensate for extreme hypoxia to a degree that mitigates reduction in condition of females or a decrease in survivorship of both genders, and that permits continual reproduction in a laboratory setting.

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CHAPTER 1

PHYSIOLOGICAL DIVERSITY: AN INTEGRATIVE STUDY OF RESPONSE TO HYPOXIA IN THE LIVEBEARING SAILFIN MOLLY (*POECILIA LATIPINNA*)

A central issue in physiological ecology is an understanding of how animals respond physiologically to natural variation in the physical environment and how this influences both the distribution and abundance of populations and the evolutionary consequences of physiological response. The physical environment has a major influence on the distribution and movement of organisms. For fishes, the availability of dissolved oxygen is one abiotic factor that can exert a strong selective force, affecting habitat quality, growth, survival and reproduction (Kramer, 1983a, 1987; Saint-Paul and Soares, 1987; Chapman and Liem, 1995; McKinsey and Chapman, 1998; Secor and Gunderson, 1998; Chapman et al., 1999; Pichavant et al., 2000). Hypoxia (oxygen scarcity) is a common challenge to survival in many aquatic systems, and has led to behavioral, morphological, anatomical, physiological, and biochemical adaptations. Some of these responses can be activated over the short-term (minutes, hours, days), while others are genetically fixed and/or developmentally plastic. Although much effort has been directed towards describing adaptations to deoxygenation in fishes, little is understood about how morphological, behavioral and/or physiological responses to hypoxia interact or the potential evolutionary consequences of hypoxia adaptation. In this dissertation, I use an integrative approach to address these issues by combining studies of behavioral, morphological, and physiological responses to hypoxia within and between populations. In this introductory chapter, literature on spatial and temporal patterns of hypoxia is

reviewed to illustrate the widespread occurrence of oxygen scarcity in natural and anthropogenically-altered waters. Adaptations of fishes to hypoxia stress are then reviewed to demonstrate the diversity of physiological, behavioral, and morphological response, the costs of hypoxia exposure, and the potential consequences for faunal diversification. Finally, study objectives are outlined and the focal organism, the sailfin molly, *Poecilia latipinna* is introduced.

Hypoxia: Spatial and Temporal Patterns in Aquatic Systems

All fish require oxygen for long-term survival. However, the physical properties of water make oxygen uptake a challenge even at high dissolved oxygen levels (normoxia). Sixty times more viscous than air, water is 800 times denser, and holds 1/30th the oxygen content of air at saturation (Nikinmaa and Salama, 1998; Dejours, 1994). In addition to the constraints of oxygen uptake imposed by the physical properties of water, there are many systems where water may not remain saturated with oxygen, leading to hypoxia. Without adequate mixing at the surface or light limitation of photosynthetic production of oxygen, faunal respiration can deplete the oxygen of a body of water. Systems characterized by such factors include heavily vegetated swamps (Carter and Beadle, 1931; Carter, 1934, 1955; Chapman and Liem, 1995; Chapman et al., 1998), flooded forests (Gessner, 1961; Kramer et al., 1978), floodplain lakes and ponds (Welcomme, 1979; Junk et al., 1983), profundal waters of lakes and ponds (Wetzel, 1975; Lewis and Weibezahn, 1976; Rai and Hill, 1981), ice-covered northern lakes (Blazka, 1958; Holeton, 1979), salt marshes (Chapter 5), some bathyspheric coastal waters (Hughes, 1973), spring heads (Odum and Caldwell, 1955; McKinsey and Chapman, 1998), and some highly colored waters (Crisman and Chapman, 1999).

Hypoxia occurs in high altitude lakes as well (e.g., Lake Titicaca of the Andes), where dissolved oxygen concentration may be low even at saturation (Hughes, 1973).

Oxygen scarcity (hypoxia) in aquatic habitats can be both naturally occurring and anthropogenically induced. Municipal wastes and fertilizer run-off can increase nutrient levels within adjacent water bodies significantly, causing bacterial, algal, and/or macrophytic blooms, depleting the water of dissolved oxygen (Jensen et al., 1993). These conditions are not confined to inland waters; nutrient enrichment can lead to hypoxic conditions in marine habitats as well. An increasing number of coastal and estuarine systems, such as the Chesapeake Bay, now commonly experience hypoxic bottom waters (Seki et al., 1974; Harper et al., 1981; Dethlefsen and von Westernhagen, 1983; Officer et al., 1984; Rosenberg, 1985; Seliger et al., 1985; Mackiernan, 1987; Diaz et al., 1992).

Variation in dissolved oxygen availability may occur on a variety of spatial and temporal scales. Photosynthetic processes of algae and/or macrophytes in eutrophic lakes or small exposed pools often lead to diel variation in dissolved oxygen levels from hyperoxia (supersaturation of oxygen) at midday to near anoxia at night (Congleton, 1980; Kramer et al., 1978; Chapman and Chapman, 1993; Jensen et al., 1993), while pools characterized by little mixing under dense forest cover may be consistently hypoxic during periods of isolation (Chapman and Kramer, 1991a). Tide pools are often characterized by short cycles of isolation and hypoxia followed by inundation with air-saturated water associated with phases of the tide (Congleton, 1980; Innes and Wells, 1985). Strong seasonal variation in dissolved oxygen associated with seasonal fluctuations in rainfall, mixing, incident light, and water temperature occurs in many

systems. In intermittent streams, habitats may shift from fast flowing, well-oxygenated habitats in rainy seasons, to small isolated hypoxic pools when rain becomes scarce (Chapman and Kramer, 1991b). Spatial variation in oxygen is evident as strong vertical gradients in many deep lakes (Rai and Hill, 1981) and in horizontal gradients in lakes with marginal areas covered by floating vegetation (Bonetto et al., 1969; Saint-Paul and Soares, 1987).

Mechanisms Compensating for Hypoxia in Fishes

Air-breathing Fishes

Oxygen scarcity has produced various evolutionary responses by fishes. Air breathing has evolved many times in euteleosts and represents independent specializations to aquatic hypoxia in several phylogenetically distinct lineages (Liem, 1980; Kramer, 1987; Graham, 1997). Specialized organs for aerial respiration occur in several swamp species, including lungfishes (Johansen, 1970; Datta-Munshi and Hughes, 1992b; Graham, 1997). These elaborate organs adapted to aerial respiration have significant vascular modifications associated with them (Olson, 1994). For example, the lungfish *Lepidosiren* spp., has vascular modifications that separate oxygenated and deoxygenated blood at the heart, shunting deoxygenated blood through its “lung” (Olson, 1994). Alimentary gas exchange is common in the families Cobitidae and Callichthyidae (Datta-Munshi and Hughes, 1992a; Graham, 1997). In these fishes, gulped air is passed through the alimentary canal for gas exchange through vascularized patches in the stomach (e.g., *Ancistrus anisitsi*, *Plecostomus plecostomus*) or intestine (e.g., *Hoplosternum* spp., *Misgurnus fossilis*), exiting either through the mouth or anus (Datta-Munshi and Hughes, 1992a). A number of intertidal fishes have structurally reinforced

gills adapted for aerial respiration (e.g., many Gobiesocidae and some Clinidae, Datta-Munshi and Hughes, 1992a). Gas bladders adapted to function in oxygen uptake are found in primitive fishes including some members of the Osteoglossomorpha, Elopomorpha, and Euteleostei. For example, gar, *Lepisosteus* spp., have a gas bladder with a direct (physostomus) alimentary connection that allows them to gulp air into the gas bladder for oxygen uptake (Datta-Munshi and Hughes, 1992a). Although there are many different adaptations to aerial respiration in fishes, air-breathing fishes are comparatively rare, comprising only 2% of known fish species (Graham, 1997), and the great majority of fishes living in hypoxic waters are non-air breathers.

Physiological and Morphological Responses to Hypoxia in Non-air breathers

Fishes relying on aquatic respiration in hypoxic environments have developed many strategies to increase oxygen transfer from the environment to their tissues and to evade problems associated with decreased dissolved oxygen availability. These strategies are not necessarily unique to non-air-breathing fishes, but are well developed in many air-breathers that experience hypoxia. Short-term physiological responses, related to biochemical modifications, provide flexibility to deal with variable oxygen habitats and include adjustment of gill perfusion, regulation of the proportions of different hemoglobin (Hb) fractions, adjustment of intraerythrocytic nucleotide triphosphate (NTP) concentrations or ratios, changes in hematocrit (Hct) and hemoglobin concentration ([Hb]), and metabolic depression (Beamish, 1964; Riggs, 1970; Hughes, 1973; Lykkeboe et al., 1975; Johansen and Weber, 1976; Johansen et al., 1978; Hughes, 1981; Jensen, 1991; Laurent and Perry, 1991; Frische and Nilsson, 1993; Jensen et al., 1993; Perry and McDonald, 1993; Val and De Almeida-Val, 1995). Under hypoxic conditions, increased

ventilation rate and/or stroke volume is a frequent response (Holeton and Randall, 1967; Davis and Cameron, 1970; Itazawa and Takeda, 1978; Holeton, 1979; Rantin et al., 1992) that increases the rate of water flow over the gills and maximizes the blood/water PO_2 gradient. Hypoxia can also induce bradycardia, as well as an increase in cardiac stroke volume. These factors tend to cancel each other out and produce little change in cardiac output. However, an increase in stroke volume tends to increase arterial blood pressure, which has a role in lamellar recruitment. A fish may be able to employ more of its distal lamellae both by shunting blood from basal channels to more central channels within the open lamellae (Frische and Nilsson, 1993; Perry and McDonald, 1993). Adrenergic control of vasoconstriction and dilation at afferent and efferent arterioles of the secondary lamellae also increases recruitment of secondary lamellae of each gill arch (Aota et al., 1990; Kinkead and Perry, 1991). The rate of oxygen uptake can be increased by this elevated perfusion of lamellae (maximizing surface area for exchange) and by increasing ventilation rate (Nilsson, 1986; Frische and Nilsson, 1993; Perry and McDonald, 1993). In rainbow trout, *Oncorhynchus mykiss*, acclimation to hypoxia reduces the thickness of the epithelium of the secondary lamellae, functioning to decrease the diffusion distance of the blood-water barrier at the gills and further facilitate oxygen uptake (Soivio and Tuurula, 1981; Tuurula et al., 1984; Perry and Wood, 1989).

Other gill morphometric characteristics, such as primary lamellar length and secondary lamellar density, may reflect response to long-term chronic hypoxia. Many fish adapted to chronic hypoxia have longer primary lamellae and higher secondary lamellar density and/or area than is predicted based on body size alone (Galis and Barel,

1980; Fernandes et al., 1994; Chapman and Liem, 1995; Chapman et al., 1999, 2000; Chapman and Hulen, in press).

Blood oxygen-transport in most teleosts is dependent on hemoglobin and is increased by adjusting the affinity of hemoglobin for oxygen, increasing the number of erythrocytes in circulation, and/or increasing hemoglobin concentration (Hughes, 1973; Johansen et al., 1978; Soivio et al., 1980; Jensen, 1991). Blood oxygen-capacity changes (increased hemoglobin concentration and hematocrit) in response to hypoxia have been reported for several species including rainbow trout (Tetens and Lykkeboe, 1981; Hughes and Kikuchi, 1984; Tun and Houston, 1986; Boutilier et al., 1988; Wells and Weber, 1991); sheepshead minnow and sailfin molly (Peterson, 1990), Crucian carp and wild goldfish (Bel'Chenko and Kel, 1992), some Amazonian fishes (Graham, 1985; Val et al., 1992), Antarctic borch (Wells et al., 1989), and yellowtail (Yamamoto et al., 1985). However, these blood capacity changes are often modest and some species do not consistently show a change (Marinsky et al., 1990). An increase in blood oxygen-affinity of hemoglobin is frequently observed in response to hypoxia and is mediated by increased pH (Haldane effect), decreased erythrocytic concentration of organic phosphates, or variation in the ratio of one nucleoside triphosphate compound to another (e.g. GTP/ATP, Bartlett, 1978; Weber and Lykkeboe, 1978; Johansen et al., 1978; Tetens and Lykkeboe, 1981; Val et al., 1992). Increased gill ventilation results in decreased serum bicarbonate and CO₂ levels, thus acting to increase blood pH (Perry and McDonald, 1993) and facilitating increased hemoglobin-oxygen affinity. In addition, adrenergic activation of the sodium proton-exchange pump of the red blood cell increases hemoglobin-oxygen affinity by increasing intraerythrocytic pH and cell volume (Thomas

and Hughes, 1982; Tetens and Christensen, 1987; Nikinmaa and Salama, 1998).

Teleosts often have complex hemoglobin systems, the components of which sometimes differ markedly in their sensitivity to intraerythrocytic phosphates and pH, and those can be important factors in hypoxia tolerance (Fyhn et al., 1979; Riggs et al., 1979; Perez and Rylander, 1985; Marinsky et al., 1990; Perry and McDonald, 1993).

An additional strategy used by fishes in conjunction with other physiological adjustments is to lower metabolic rate. A lower metabolic rate reduces the impact of a lower oxygen tension, but has the disadvantage of reducing aerobic metabolism and the amount of energy available for many biochemical processes. Nonetheless, a relatively low metabolic rate seems to be characteristic of several swamp-dwelling fishes inhabiting chronically hypoxic waters (Chapman and Chapman, 1998, Rosenberger and Chapman, 2000).

The majority of fish species can be described as metabolic oxygen regulators. These fishes are capable of maintaining a constant metabolic rate over a range of oxygen tensions. The minimum oxygen tension required to maintain a constant metabolic rate is defined as critical tension (P_c). Below this value, metabolism decreases linearly with oxygen tension. Fry (1957) initially recognized the value of P_c as an important species' characteristic. Critical tension can be affected by diverse morphological and physiological responses to hypoxia, and is therefore a very useful measure of adaptation, acclimatization, or acclimation to hypoxia.

When oxygen uptake does not meet metabolic demands, some fish are able to utilize anaerobic metabolism to cope with extreme hypoxia effectively. However, for vertebrates there are severe problems associated with long-term dependence on anaerobic

metabolism. Compared to aerobic metabolism, anaerobic metabolism is an inefficient means of yielding energy, (4 moles ATP compared to 36 moles ATP/ mole glucose); it yields few of the essential intermediaries for tissue maintenance and growth, and produces toxic end products (e.g., lactic acid) (Eckert and Randall, 1983). Accumulation of lactate alters pH, causing the cells to become more acidic, and threatens protein structure and function. Hypoxia-acclimated fishes may be able to produce enzymes more tolerant of pH changes associated with lactic acid build up, and lactate can be removed from circulation by increased levels of gluconeogenesis or lipogenesis (Blazka, 1958; Johnston, 1975; Smith and Heath, 1978; Van Den Thillart, 1982; Hochachka, 1993). One remarkable example is crucian carp, *Carassius carassius*, which engage in anaerobic metabolism to survive months of anoxia in frozen Siberian lakes and appear to regulate lactate accumulation effectively through lipogenesis (Blazka, 1958).

Behavioral Responses to Hypoxia

Behavioral responses provide additional flexibility to deal with rapid variation in dissolved oxygen. Fish may attempt to avoid hypoxic areas through microhabitat selection (Breitburg, 1994; Chapman and Liem, 1995) or compensate for low oxygen levels through aquatic surface respiration (ASR; Kramer and Mehegan, 1981). The surface film of water contains high concentrations of oxygen, and many fish take advantage of this by skimming the surface film and passing this oxygen rich water over their gills, a behavior referred to as surface skimming or ASR. Aquatic surface respiration is more efficient than simply increasing ventilation under extreme hypoxia (Kramer, 1983a; Kramer and Mehegan, 1981). In addition, some fishes increase the efficiency of oxygen uptake during ASR by swimming continuously across the surface

(Chapman et al., 1994, 1995; Chapman and Liem, 1995; Rosenberger and Chapman, 2000). This "ram-assisted" ASR (Chapman et al., 1994) may pass more oxygenated surface water across the gills than would be achieved by active gill ventilation alone and may contribute to hydrodynamic lift. This increased lift may minimize the energy required to remain in contact with the surface, especially for fish without gas-bladders, such as benthic species. In addition, the use of buccal bubble-holding, as observed in several fishes, may increase the efficiency of ASR by serving as a buoyancy compensation mechanism and/or by increasing the oxygen content of the water passing over the bubble (Burggren, 1982; Gee and Gee, 1991).

The Interaction of Pollutants and Hypoxia Response

Pollutants such as acid rain, organic xenobiotics, heavy metals, and/or suspended solids can hamper a fish's ability to cope with reduced dissolved oxygen levels and exacerbate the effects of the increasingly common threat of hypoxia in coastal and freshwater systems. (Hughes, 1973, 1981; Holeton, 1979; Evans, 1987; Jensen et al., 1993). Acid rain frequently lowers water pH to levels damaging to gill tissues, threatening internal pH regulation, and interfering with respiration (Hughes, 1981). Severe gill morphological changes associated with exposure to organic xenobiotics may also have a detrimental effect on oxygen uptake during simultaneous exposure to hypoxia (Evans, 1987). Heavy metals such as cobalt, zinc, and nickel can reduce diffusion capacity of the gills by causing the epithelium of the secondary lamellae to thicken, increasing the diffusion distance of oxygen through the blood-water barrier (Evans, 1987; Jensen et al., 1993). Chemical pollutants may also cause anaemia, either through bleeding or interference with hemoglobin binding properties (Hughes, 1981). For

example, cadmium acts to reduce blood oxygen capacity by binding irreversibly with hemoglobin. The neurological effects of some chemical pollutants, such as zinc, interfere with cardiac and ventilatory regulation and coughing mechanisms (Hughes, 1981). Fish often cough in the presence of suspended solids that can precipitate on the gills and significantly reduce surface area available for respiration (Hughes, 1981; Jensen et al., 1993). Understanding the interaction of physiological and behavioral response to hypoxia and the potential costs of different strategies is critical for predicting response to pollutants.

Potential Costs of Hypoxia Adaptation

The functional significance of physiological adaptations to hypoxia exposure is that such adaptations permit fishes to survive in environments that are subject to periodic or chronic hypoxia. Benefits of living in such harsh environments may include lower levels of competition for food, shelter, or predation. However, exposure to hypoxia may ultimately reduce fitness. Studies linking hypoxia exposure to fitness-related characters have shown growth depression in long-term experiments (Scott and Rogers, 1981; Bejda et al., 1992). Costs of short-term acclimation responses are less well known but may involve growth depression, reduced fecundity, or smaller offspring, changes in condition, increased predation rate, etc. In addition to imparting potentially high fitness costs, many of the physiological responses described above do not occur rapidly enough to meet energetic demands when environmental oxygen availability drops at a very high rate (Hochachka and Somero, 1984; Wootton, 1990). Behavioral adaptations (e.g., ASR) offer a quick response to hypoxic stress but can incur very high costs. For example, under extreme hypoxia, many fishes spend greater than 90% of their time at the surface

using ASR (Chapman et al., 1994, 1995; Chapman, 1995; Olowo and Chapman, 1996). This clearly limits time that can be directed to foraging, reproduction, and other activities. Increased time spent conducting ASR imposes a high energetic cost, and may also expose the fish to increased potential risk of aerial predation (Kramer et al., 1983). Because of the high costs of ASR, one might predict this immediate behavioral response to hypoxia to be reduced or replaced by physiological mechanisms within hours or days of exposure. However, little is known about the interaction between behavioral and physiological traits in response to hypoxia exposure.

Costs of hypoxia adaptation may also vary with reproductive strategy and state. For example, by increasing costs of parental care in some oviparous fishes (e.g., egg fanning) hypoxia might limit reproductive success or constrain habitats available to brooding parents. Viviparity may allow reproducing females flexibility in coping with hypoxia. A female may behaviorally (through avoidance of hypoxic waters) or physiologically (through increases in blood oxygen capacity) attempt to compensate for hypoxic conditions. However costs of hypoxia for gestating females may be high. Accumulating evidence indicates that actively developing tissues of embryos have a higher mass specific oxygen requirement than maternal tissues. In several Scorpaenid species of the genus *Sebastes*, mass-specific metabolic rates increases in late gestation (Boehlert and Yoklavich, 1984; Boehlert et al., 1986; Dygert and Gunderson, 1991). The increase in metabolic demand of embryos during late development may critically stress oxygen uptake mechanisms under hypoxic conditions, significantly reduce their metabolic scope of activity, and potentially alter their respiratory behavior. No published studies have examined the potential costs of viviparity under hypoxia. However, during

gestation, increased metabolic demand of a brood may result in higher behavioral allocation to ASR under hypoxia exposure, thus potentially increasing associated risk of aerial predation (Kramer et al., 1983).

Hypoxia and Interdemic Variation

Given the potential costs of hypoxia adaptation, strong selection pressure for hypoxia tolerance in oxygen-scarce habitats and relaxation of selection pressure for low oxygen tolerance in well-oxygenated habitats may lead to interdemic variation among populations with broad habitat ranges.

For air-breathing fishes, hypoxic waters are not likely to limit dispersal. However, for non-air breathers that cannot tolerate hypoxia, oxygen-scarce habitats such as swamps or other wetlands may serve as an isolating mechanism. For non-air breathers that can survive in hypoxic waters, habitat use and dispersal may still be limited by dissolved oxygen availability and oxygen uptake efficiency. This may result in geographical variation among populations from sites that differ in dissolved oxygen availability, particularly with respect to respiratory traits. For example, Chapman et al. (1999) quantified the relationship between total gill filament length and dissolved oxygen concentration (DO) for the cyprinid *Barbus neumayeri* from six sites in a river drainage in Uganda that differed in DO availability. Total gill filament length increased as DO decreased, indicating interdemic variation in a morphological trait that correlated with dissolved oxygen availability. Further study revealed differences in respiratory behavior between *B. neumayeri* from swamps and those from open waters in the same river system (Olowo and Chapman, 1996). This interdemic variation may derive from either the

geographical origins of the populations or the developmental and environmental histories of the individuals within the populations.

From the perspective of an ecological physiologist, there are still some major obstacles that preclude a thorough understanding of the link between physico-chemical stressors and interdemic variation. These obstacles must be addressed before examination of the source of interdemic physiological diversity can begin. One major issue is the fact that physiological variation occurs at multiple hierarchical levels, i.e., short-term acclimation or acclimatization response, developmental plasticity, genetic variation, and/or their interaction. Historically, there has been an emphasis on variation between species and acclimation effects on individuals. Studies of interdemic variation in fishes are uncommon and generally have ignored the interaction between acclimation effects and fixed or developmental interdemic effects.

Study Objectives

This study was designed to understand mechanisms compensating for hypoxia in the live-bearing sailfin molly, *Poecilia latipinna* (Poeciliidae) with focus on behavioral and physiological responses, fitness consequences of hypoxia adaptation, and implications of hypoxia for interdemic variation. Focus was primarily on *P. latipinna* collected from a periodically hypoxic salt marsh in Cedar Key, Florida, but fish collected from well-oxygenated waters of the Santa Fe River were also included in interdemic comparisons. Chapter 2 examines whether oxygen requirements of gravid female mollies varies with developmental stage of a gestating brood, and whether associated energetic demand increases time allocated to ASR under hypoxic stress. The role of short-term behavioral and physiological responses in permitting *P. latipinna* the flexibility to deal

with extreme hypoxia are examined in Chapter 3. Potential costs of hypoxia acclimation are examined by measuring fitness-related characters (reproduction, fish condition, survivorship) for fish acclimated to low and high dissolved oxygen levels. To detect the interactive nature of acclimation response, an integrative approach was used that combines studies of blood characters, metabolic rate, critical oxygen tension, behavior, reproduction, fish condition, and survival. In this dissertation I use the term “integrative” to describe the consideration of diverse elements (e.g., behavioral, physiological) to provide a more holistic description of the response than would be available from the examination of a single factor. I define acclimation in this study as measured compensatory changes developed over time in the laboratory in response to varying a single environmental character. In Chapter 4, the scale of analyses was increased by quantifying physiological diversity in hypoxia compensation between two populations of *P. latipinna*, one population from a periodically hypoxic environment, and another population from a normoxic environment. Chapter 5 is an overview of the spatial and temporal patterns of dissolved oxygen concentration in the periodically hypoxic waters of the Cedar Key marsh. Major conclusions of the study are summarized in Chapter 6.

Characteristics of *Poecilia latipinna*

Poecilia latipinna (the sailfin molly) is an ovoviviparous species of the teleost family Poeciliidae, a monophyletic group of 22 genera and 12 subgenera distinguished from other cyprinodontiforms by derived characteristics of the head and gonopodium (Parenti and Rauchenberger, 1989; Table 1-1). In this family of about 190 species, all but one (*Tomeurus gracilis*) are livebearing (Constanz, 1989). Poeciliids produce relatively

small numbers of precocial young (Thibault and Schultz, 1978). At birth, the young have well-developed gills with secondary lamellae, a gas bladder, and a functional acousticolateralis system (Scrimshaw, 1945).

Among these livebearing species, there is a continuum of lecithotrophic to matrotrophic nutrient provisioning to the brood. Lecithotrophic species rely solely on yolk stores to fuel development (e.g., *Poecilia reticulata*, Meffe and Snelson, 1989), while matrotrophic species provide extra nutrients to developing embryos, after egg formation (e.g., *Xiphophorus* spp., Scrimshaw, 1945; Wourms, 1981). Embryos of lecithotrophs are unable to maintain original weight following fertilization, while matrotrophic species either maintain or increase post-fertilization embryonic weight (Scrimshaw, 1945). Although considered primarily lecithotrophic, *Poecilia latipinna* may be able to alter matrotrophic investment under certain environmental conditions (Trexler, 1985, 1997).

In fishes, viviparity can be considered a form of parental care and offers the developing embryos increased protection from predation, thermal, or oxygen stress (Constanz, 1989; Wootton, 1990). During gestation there are two essential maternal functions: to provide oxygen to and remove wastes from the brood. With embryos packed tightly in the ovary of the female during gestation, crowding may pose challenges to aerobic respiration and nitrogen excretion (Guillette, 1987). It has been postulated that stress hormones produced by a brood when these requirements are not met may stimulate early parturition in *P. latipinna* (Guillette, 1987). Although this species is often found in warm waters subject to periodic hypoxia, the effects of environmental hypoxia on reproduction in this species are not known.

Native throughout the coast of the Gulf of Mexico, as well as in inland freshwaters of Florida (Simanek, 1978; Burgess, 1980; Lee et al., 1980; Trexler, 1986), sailfin mollies have also become established in Central America, Singapore, Australia, New Zealand, the Philippines, Guam, Hawaii, Canada, Arizona, California, and Nevada (Arthington and Lloyd, 1989). Capable of storing sperm, a single gravid female is all that is necessary to establish a population (Constanz, 1989). The species is euryhaline (0-80 ppt; Nordlie et al., 1992) and occurs in rivers, wetlands, marshes, the littoral zone of eutrophic lakes, sewage effluent canals, and estuaries (Burgess, 1980). Generally tolerant of extreme environmental conditions and being an excellent colonizer, the sailfin molly and other members of the Family Poeciliidae often occupy fringe habitats (Courtenay and Meffe, 1989; Meffe and Snelson, 1989).

As vertebrates with relatively short reproductive cycles and broad geographical distribution, poeciliids have been valuable in the study of the evolution of life-history traits (e.g., *P. reticulata*; Reznick and Bryga, 1987; Endler, 1980; genus *Xiphophorus*; Kallman, 1983, 1984; Borowski, 1987). Several examples of unisexual species that reproduce by gynogenesis, have been discovered (e.g., *P. formosa* hybrids and the genus *Poeciliopsis*), and their characteristics have been useful in testing assumptions of evolutionary models (Balsano et al., 1989; Wetherington et al., 1989). Research on sailfin mollies has focused on reproductive behavior and ecology (Baird, 1968, 1974; Snelson and Wetherington, 1980; Farr and Travis, 1986; Farr et al., 1986; Travis and Woodward, 1989; McManus and Travis, 1998), reproductive physiology (Theissen and Sturdivant, 1977; Sheinbaum, 1979; Grier, 1980; Trexler, 1985; Smith, 1986; Snelson et al., 1986; Smith 1988), and osmoregulation (Evans, 1975; Gustafson, 1981; Nordlie et

al., 1992). In addition, there has been significant research on genetic variation or phenotypic plasticity in life history traits (Farr and Travis, 1989; Large, 1985; Luckner, 1979; Snelson, 1982, 1984, 1985; Travis and Trexler, 1987; Travis et al., 1989; Trexler, 1988). Sailfin mollies were selected for this study because of their tolerance to extreme hypoxia, short reproductive cycles, and broad geographic distribution and a diverse range of habitats. These are criteria that offer an excellent opportunity to examine both behavioral and physiological mechanisms and interdemc variation in ability to cope with hypoxia.

Table 1-1. Taxonomic classification of poeciliine fishes.

Order Cyprinodontiformes

Suborder Cyprinodontoides

Family Poeciliidae (190 sp)

Supertribe Tomeurini

Supertribe Poeciliini

Tribe Poeciliini

Genus *Alfaro*

Genus *Poecilia*

Subgenus *Poecilia*

Subgenus *Lebistes*

Subgenus *Pamphorichthys*

Subgenus *Limia*

Subgenus *Odontolimia*

Genus *Priapella*

Genus *Xiphophorus*

Tribe Cneusterodontini

Tribe Scolichthyini

Tribe Gambusiini

Tribe Girardiniini

Tribe Heterandrinii

Tribe Xenodexiini

Modified from Parenti and Rauchenberger, 1989

CHAPTER 2

THE EFFECT OF GESTATIONAL STATE ON OXYGEN CONSUMPTION AND RESPONSE TO HYPOXIA IN THE SAILFIN MOLLY (*POECILIA LATIPINNA*)

Introduction

The most common mode of reproduction in teleosts is oviparity (egg laying). Many fish protect their developing young after spawning, thereby increasing the percent survival of their brood (Keenleyside, 1979). This oviparous parental care may require selecting suitable nesting sites (with adequate temperature, dissolved oxygen levels, substrate type, and structural cover), as well as nest building and guarding young. This protective behavior exacts a significant cost to the parents, who may refrain from feeding for days or weeks during the vulnerable period of offspring development (Wootton, 1990).

Mouthbrooding is a behavior that allows parental protection of young while allowing flexibility to readily move the brood to more suitable environments under fluctuating conditions of habitat quality (i.e., temperature, dissolved oxygen levels, predation pressure). The next step along a continuum of parental care strategies is ovoviviparity. This mode of reproduction involves internal fertilization and retention of embryos in either the ovary or uterus until they reach a more advanced (precocious) and less vulnerable stage of development. The cost of protecting offspring in live-bearing species can include increased work for the cardiac system, increased branchial ventilation rate, and increased cost of osmoregulation (due to increases in ventilation rate). Further

along this continuum, some species have evolved specialized tissues analogous to placentae to provide nutrients to developing broods, a strategy referred to as viviparity (Thibault and Schultz, 1978; Blackburn et al., 1985; Constantz, 1989). During gestation, the maternal system must provide oxygen to and remove metabolic wastes from the embryo at the site of the follicle, and therefore must respond to changes in metabolic oxygen requirements of her developing brood (Thibault and Schultz, 1978; Blackburn et al., 1985). Early in development, most of the embryonic material (water and yolk) does not respire. As an embryo develops, metabolic activity increases, so that just prior to parturition, more than 70% consists of actively metabolizing material (Kamler, 1992). The actively developing tissues of the embryo, just prior to hatching, may have a higher mass specific oxygen requirement than maternal tissues and temporarily increase the total routine oxygen requirement of a live-bearing female (Boehlert and Yoklavich, 1984; Boehlert et al., 1986; Dygert and Gunderson, 1991). The following equation summarizes the metabolic oxygen requirements (VO_2) of a gestating female (DeMarco, 1993):

$$VO_2 \text{ total} = VO_2 \text{ nonreproductive} + VO_2 \text{ litter maintenance} + VO_2 \text{ embryos}$$

Unfortunately, it is extremely difficult to measure the separate components contributing to the total metabolic oxygen requirement of a female during gestation (DeMarco, 1993). Therefore, most estimates of the metabolic rate of developing embryos in fishes have been done on either the embryos of oviparous species (Lasker and Theilacker, 1962; Holliday et al., 1964; DeSilva and Tytler, 1973; Houde and Scheckter, 1983; Morioka, 1985; Oikawa and Itzawa 1985, 1992, 1993; DeSilva et al., 1986; Walsh et al., 1989; Oikawa et al., 1991; Oozeki and Hirano, 1994) or using *in vitro* techniques on live-bearing species (Moser, 1967; Webb and Brett, 1972; Boehlert and Yoklavich,

1984; Berglund et al., 1986; Boehlert et al., 1986; Dygert and Gunderson, 1991).

In the viviparous, primarily marine, genus *Sebastes*, mass-specific oxygen consumption of embryos *in vitro* increases with developmental stage either linearly (*S. melanops*, Boehlert and Yoklavich, 1984) or exponentially (*S. schlegeli*, Boehlert et al., 1986; *S. caurinus*, Dygert and Gunderson, 1991). Such *in vitro* experiments suggest an increase in oxygen consumption of embryos with development. However, these studies have been criticized because the ambient oxygen levels used during the measurements were higher than generally found in intraovarian fluids, and the embryos may be more active under the stressful *in vitro* procedures (Boehlert et al., 1991).

Comparative measurements of the respiration rate of gestating and non-gestating fish provide a second method for examining changes in mass specific metabolic rate with development. The significantly elevated respiration rates of gestating female *S. schlegeli* were indicative of an increase in oxygen consumption by embryos as well as an increase in oxygen consumption by the maternal system (Boehlert et al., 1991). The same pattern has been found in other live-bearing ectotherms such as the ovoviviparous lizard, *Sceloporus jarrovi* (Guillette, 1982; DeMarco, 1993)

An increase in metabolic demand of a brood during development would not only be an added energetic cost, but may also increase the risk of predation to a gestating female in hypoxic conditions by significantly reducing metabolic scope of activity and potentially altering respiratory behavior. Under extreme hypoxia, many non-air-breathing fishes use aquatic surface respiration (ASR; pumping the oxygen-rich microfilm at the surface of water through gills) to increase oxygen uptake (Kramer and Mehegan, 1981; Kramer and McClure, 1982). This behavior incurs a significant energetic cost as well as

an increased risk of aerial predation (Kramer, 1983b; Kramer et al., 1983). The increased metabolic demand of a brood during gestation may result in a higher behavioral allocation to ASR.

The sailfin molly (*Poecilia latipinna*) is a small live-bearing species distributed throughout the coast of the Gulf of Mexico and Florida (Simanek, 1978; Lee et al., 1980; Trexler, 1986). The species occupies a wide range of habitats and reproduce in hypoxic waters such as shallow wetlands and stagnant pools (pers. obs.). Therefore, females must be capable of meeting the metabolic requirements of a brood throughout development even under such extreme conditions. However, whether oxygen requirements of a gravid female vary with development of a gestating brood is unknown. In addition, the effect of brood development on time allocation to ASR under hypoxic conditions has not been previously studied.

This study has two major objectives. The first is to determine whether *P. latipinna* females exhibit an increase in routine metabolic oxygen consumption during the development of their broods. The second objective is to determine whether pregnant females spend a higher proportion of time at the surface (ASR) in response to hypoxia than non-gestating females.

Methods

Metabolic Rate Experiment

Poecilia latipinna were collected from Cedar Key, Florida, in August 1996.

Three aquaria, containing three females and one male each, were maintained with a 12-h photoperiod at 25 °C and 10 ppt salinity for a period of 14 wk to capture two reproductive cycles from the females. *Poecilia latipinna* is a species that readily breeds in captivity and has a short gestation period (approximately 6 wk, at 25 °C). It is generally considered to be ovoviviparous and lecithotrophic, although there is some evidence of matrotrophic investment (Trexler, 1985, 1997). The developing brood is retained within the female's paired fused ovaries (Grier, 1973).

Fish were tattooed for individual identification (McKinsey and Chapman, 1998; Chapman et al., 1999) and acclimated to their aquaria for 2 wk prior to taking metabolic readings. Water quality was maintained using a sponge filter, and fish were fed Tetra Min ® flake food *ad libitum* once daily throughout the experiment. A closed respirometry system was used to measure oxygen consumption of individuals (males and females) weekly. Individuals were acclimated over night in 0.5 l bottles rendered opaque with duct tape. The opacity of the bottle was designed to minimize disturbance to the fish during the sampling period, thereby obtaining a resting routine metabolic rate (RMR) (Winberg, 1956; Fry, 1957). Food was withheld 24 h prior to the weekly RMR reading for each fish. The oxygen level was measured at 5-min intervals by a YSI SONDE water quality probe model 600 immersed in the bottle, and the data were logged electronically using the data collection program, PC6000. To prevent low oxygen stress to the fish, each reading was stopped, and an aerator placed in the bottle once dissolved oxygen level

reached 3.0 mg l⁻¹. Water volume in the bottle was measured, the fish was weighed, and its standard length (SL) determined prior to being returned to its aquarium. Each female's stage of gestation was estimated by back-dating from the date of her brood's parturition. In this experiment, one female never reproduced and died, apparently of senescence, after 7 wk. The period of gestation was divided into three categories: early (weeks 1 and 2), middle (weeks 3 and 4), and late (weeks 5 and 6). The average of the measurements for each stage from two complete reproductive cycles for each female was used in the analyses. The average of eight weekly metabolic readings was used for determining the male's RMR s. Mass-adjusted RMR s were used for comparison of gestation period and gender and were calculated following Ultsch (1978) with the exponent for poeciliids (0.73=b) proposed by Winberg (1961):

$$\text{VO}_2(\text{adjusted}) = (\text{mean weight of all fish used})^{b-1} (\text{observed weight})^{1-b} (\text{observed VO}_2)$$

Repeated measures analysis of variance was used to determine the effect of a female's gestational stage on her RMR. Following Potvin et al. (1990), Mauchly's criterion was used to test for the compound symmetry of the variance-covariance matrix. Repeated contrasts were used to test for differences among the gestational stages (SPSS Inc. 1997). The repeated contrast compares the mean of each stage to that of the subsequent stage. A t-test was used to compare the mean mass-adjusted RMR of males to females at each gestational stage category.

ASR Experiment

Forty mollies, collected from Cedar Key in August 1998, were marked with tattoo ink for individual identification and acclimated to laboratory conditions (25°C, 10 ppt) for 6 days. This facilitated quick identification of individual fishes during trials. Two 250 l aquaria were divided in half to produce four chambers. An attempt was made to balance the number of gravid and non-gravid females in each chamber. Females preliminarily categorized as “gravid” or “non-gravid” based on external visual inspection before being placed into each chamber of the two aquaria. For each aquarium, there was a total of 20 fish, five “gravid”, five “non-gravid” fish on each side. At the conclusion of an experiment, each female was euthanized with MS222, weighed, and measured. Each female was then dissected to determine the stage of her brood (Haynes, 1995).

Dissolved oxygen levels were steadily reduced using a microprocessor-based oxygen controller that bubbled nitrogen or air slowly into the aquaria until the specified dissolved oxygen level was reached. Fish were held at $1.0 \text{ mg l}^{-1} \text{ O}_2$ over night prior to taking behavioral readings. Behavioral data consisted of noting the individuals conducting ASR every 10 sec over a period of 3 min. This was repeated 10 times for each chamber over 2 d. The average percent of time an individual was seen at the surface was calculated. Fish were classified as non-reproductive, or reproductive if they were in any stage of gestation. Stages of gestation were not compared because some stages were not well-represented in the sample.

Linear regression was used to test for an effect of body size on percent time using aquatic surface respiration (% ASR). To test for an aquarium effect, the mean % ASR for reproductively active and reproductively inactive fish were separately compared between

the two aquaria. Because no significant aquarium effects were detected, aquaria were then combined to test for a difference in mean % ASR between reproductively active and reproductively inactive individuals.

Results

Metabolic Rate

Repeated measures ANOVA indicated a significant effect of reproductive stage on mass-corrected RMR ($F=6.00$, $p=0.013$, Table 2-1). There was no difference in mass-corrected RMR between early and mid-gestation. However, there was a significant increase during late gestation (repeated contrasts, $P<0.05$, Table 2-1). Mass-corrected RMR of male mollies did not differ from females in early stage ($t=0.731$, $p=0.241$) and mid stage ($t=0.804$, $p=0.221$, Table 2-1) of gestation. However, mean mass-corrected RMR differed from females measured during the late stage of gestation ($t=2.190$, $p=0.028$, Table 2-1).

ASR Experiment

Linear regression analyses indicated no relationship between body mass and % ASR for females gestating embryos ($r^2=0.040$, $F=0.755$, $p=0.396$) or females without embryos ($r^2=0.118$, $F=2.283$, $p=0.149$). Therefore, body mass was not used as a covariate in comparing % ASR between groups. There was no difference in the mean % ASR of non-gestating individuals between aquaria one and two (mean for aquarium one = 16.82 ± 3.01 , SE; aquarium two = 12.15 ± 3.76 , $t=0.978$, $p=0.342$). Similarly, there was no difference in the mean % ASR of gestating females between aquaria one and two (mean

for aquarium one= 43.75 ± 7.58 , SE; aquarium two= 40.7 ± 8.04 , $t=0.257$, $p=0.799$).

Therefore I combined females from both aquaria to test for a difference in mean % ASR between gestating and non-gestating females. Percent ASR was much higher in gestating females than in non-gestating females ($t=4.48$, $p<0.001$, Figure 2-2).

Table 2-1. Comparison of the mean mass-corrected metabolic rate of male and female sailfin mollies during early, middle, and late stages of gestation. Differences between males and females were evaluated using a one-tailed t-test. The metabolic rate of females increased in late gestation (repeated measures ANOVA, repeated contrasts, $P < 0.05$).

Comparison	Versus	Mean mass-corrected metabolic rate \pm se	t-value	p-value
Males		0.579 \pm 0.102	-	-
	Early Gestation	0.686 \pm 0.080	0.731	0.241
	Middle Gestation	0.693 \pm 0.077	0.804	0.221
	Late Gestation	0.891 \pm 0.076	2.19	0.028

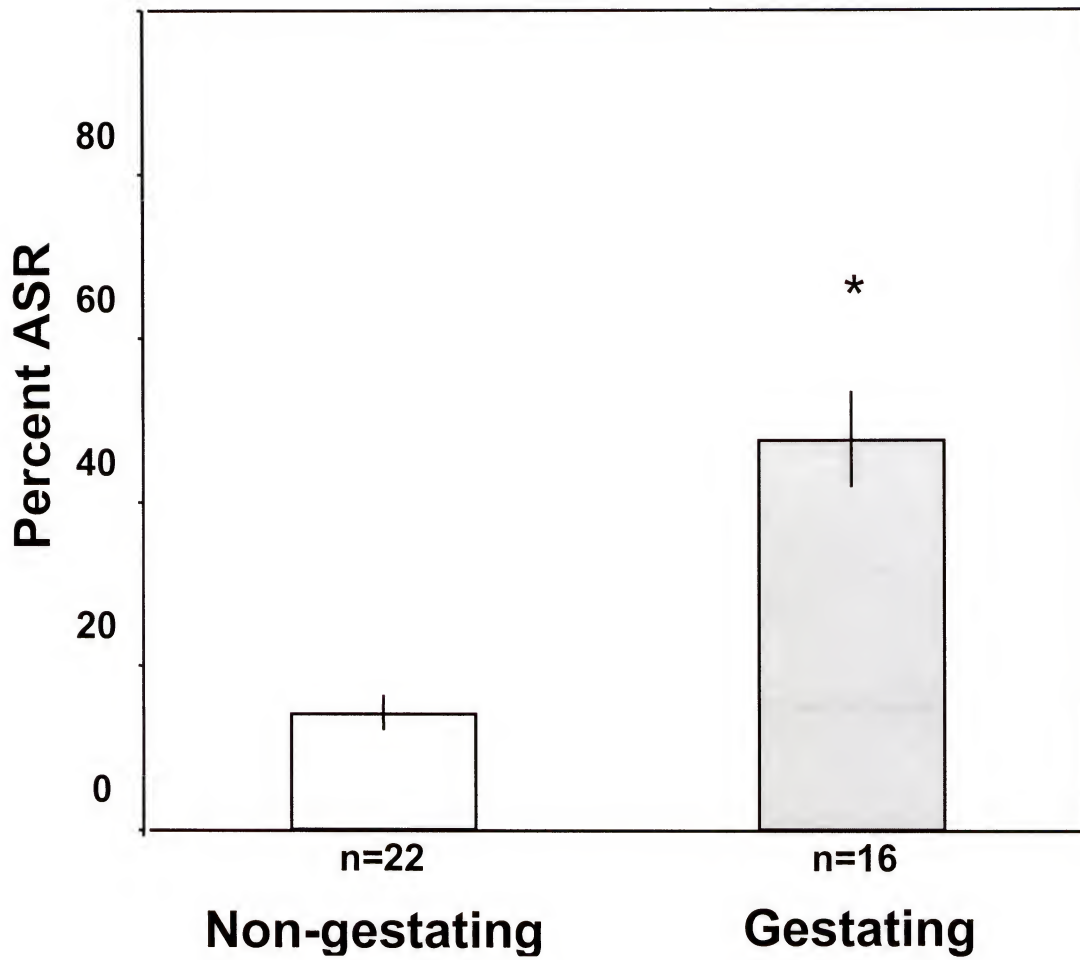


Figure 2-2. A comparison of mean %ASR (\pm SE) for non-gestating and gestating female mollies *Poecilia latipinna* collected from Cedar Key, Florida. *= $p < 0.05$.

Discussion

The results of this study agree with earlier work on other live-bearing species (*Sebastes melanops*, Boehlert and Yoklavich, 1984; *S. schlegeli*, Boehlert et al., 1986; *S. caurinus*, Dygert and Gunderson, 1991). Some live-bearing fish species have been found to increase their routine metabolic rate by as much as 68% (*Sebastes schlegeli*, Boehlert et al., 1991) just prior to parturition. Live-bearing seaperch (*Rhacochilus vacca* and *Embiotoca lateralis*) increased their metabolic rates by 53% during late gestation (Webb and Brett, 1972). The isometric relationship between mass and metabolic requirements determined for several larval fish species may be associated with the higher proportional mass of metabolically demanding tissues (the brain and kidneys) relative to less energetically active tissues (trunk and white muscle) (Oikawa et al., 1991). When almost half of a female sailfin molly's body mass constitutes her developing brood, and she may carry as many as eighty embryo's at one time (pers. obs), the additive effect of the brood's metabolic cost of organogenesis could increase total maternal metabolic oxygen requirement. In this study, the stage of gestation appears to be an important factor in determining the metabolic oxygen requirements of female *Poecilia latipinna*, leading to a significant increase in mass-specific oxygen consumption during the final third of gestation.

Most reviews of metabolic literature have not mentioned gender as a possible factor affecting metabolic rate (Cameron, 1989; Jensen et al., 1993; Jobling, 1994), and studies using gender as a factor for comparison are rare (Cech et al., 1985). Metabolic rates of male and females sailfin mollies in this study only differed during the last third of

gestation. It is likely that gender is not a significant factor affecting metabolic rate for most oviparous fishes, as unfertilized ova have a relatively low oxygen requirement (Kamler, 1992). However, a study of a gobiid fish, *Oligole acutipennis*, found that females had a 37% higher mass-specific metabolic rate than males (Geevarghese and John, 1984). While there is a metabolic cost of oogenesis, the associated oxygen requirement might be rationed out over a longer period during times of stress. For example, some live-bearing fish (e.g., *P. latipinna* and *P. reticulata*) vary their interbrood interval in response to stress or low condition, delaying fertilization or prolonging oogenesis (Snelson et al., 1986). In a study of the effects of temperature and dissolved oxygen concentration on metabolic rates of *Gambusia holbrooki*, Cech et al. (1985) found gender to be significant in only one of their nine treatment combinations. In their study, gestational state was not included as a possible factor, and may have contributed to the one anomalous result.

Trexler (1985) suggested that *Poecilia latipinna* may be a non-specialized matrotrophe, because under some environmental conditions there is no loss of embryonic dry weight during gestation. Under such conditions, it is assumed that costs associated with development are supplemented by the mother during gestation (Hoar, 1969; Wourms, 1981; Trexler 1985, 1997). The elaborate structures of the ovary probably evolved primarily for oogenesis, but on egg retention, may have been co-opted for transfer of oxygen (primarily) and (secondarily) other nutritive factors (Guillette, 1987). In a study by Foscarini (1989) *Poecilia latipinna* (unlike the oviparous fish larvae studied) developed secondary lamellae on its gills, indicating possible functionality, a few days prior to parturition. Oviparous species like the Japanese medaka (*Oryzias latipes*)

and the dwarf gourami (*Colisa lalia*) depend on cutaneous respiratory structures for respiration up to 18 days post-hatching (Foscarini, 1989). These relatively altricial oviparous species may not normally encounter hypoxia during larval development, allowing for a delay in the development of gill function. However, it has been suggested that intrafollicular hypoxia in the viviparous sailfin molly may be a normal component of parturition initiation (Guillette, 1987). Measurements of intraovarian oxygen tension of live-bearing seaperch (*Rhacochilus vacca* and *Embiotoca lateralis*) showed a decrease in tension with increasing metabolic demands of the brood, reaching a minimum of 13.7 mm Hg just prior to parturition (Webb and Brett, 1972). It is possible that the increased embryonic metabolic oxygen requirements as development progresses reduces dissolved oxygen in the lumen of the follicle, eventually stimulating an embryonic stress response. During late gestation, the follicle may have increased sensitivity to the embryonic release of adrenal corticosteroids and catecholamines, and this stress response may normally initiate parturition (Guillette, 1987). There is anecdotal evidence of a possible link between embryonic hypoxia stress response and parturition from metabolic critical tension experiments on sailfin mollies (Chapter 3). Exposure to extreme hypoxia led to premature parturition of late-stage embryos on several occasions. In contrast, females with unfertilized ova or young embryos have not been observed to abort under similar conditions. Whether functional gills contribute to the embryo's oxygen uptake efficiency to prolong gestation as intrafollicular dissolved oxygen decreases is unknown. However, the presence of functional gills at parturition may explain the survival and growth of young under chronic hypoxia (Chapter 3).

Poeciliids in general are very effective at conducting ASR, having a head morphology that appears to be well adapted to that purpose. Their supra-terminal mouth and dorso-ventrally flattened head allow access to the air-water interface without breaking the surface of the water (Lewis, 1970). Several species in the family Poeciliidae have been reported to use ASR: *Poecilia gillii* (Chapman and Chapman, 1993); *Gambusia holbrooki* (McKinsey and Chapman, 1998; Cech et al., 1985); and *Poecilia reticulata* (Kramer and McClure, 1982; Weber and Kramer, 1983). Populations faced continuously with low oxygen conditions often alternate other activities, such as feeding and reproduction, with periodic bouts of ASR (Weber and Kramer, 1987; Chapman and Chapman, 1993). However, there are high costs to ASR. Under extreme hypoxia, many fish species that use ASR have been observed to spend over 90% of their time at the surface (Gee et. al., 1978; Kramer and Mehegan, 1981; Kramer and McClure, 1982; Saint-Paul and Soares, 1987, Chapman and Liem, 1995; Olowo and Chapman, 1996).

In this study, gestating females spent approximately 20% more time conducting ASR than non-gestating females, further supporting a brood-related increase in oxygen demand. Increase time spent on ASR may directly affect maternal predation risk in low-oxygen conditions. In some fishes, the risk of aerial predation increases with time spent at the surface conducting ASR (Kramer et al., 1983). Additionally, egrets and herons appear to prefer larger sailfin mollies (Trexler et al., 1994). The larger silhouette of a gravid female at the surface would probably be more visible to aerial predators than the same female in a non-reproductive state. Females near parturition may be more vulnerable to aerial predators, spending more time at the surface at a time when they would already be a preferred prey item of some wading birds. In the field, one might

anticipate behaviors by gestating females to reduce the risk such as selection of microhabitats with elevated levels of dissolved oxygen or areas that reduce aerial predation risk such as vegetated cover. Differential recruitment of organs throughout ontogeny is considered to be an important factor in the biphasic pattern in metabolism-mass scaling observed for many fish species (Post and Lee, 1996). It is possible that if groups in this ASR experiment could have been partitioned with finer resolution (by the embryonic stage of organogenesis or recruitment of organs), the differences in time spent conducting ASR by females in different gestational states may have been determined with more resolution. Future studies linking gestational state with field micro-distribution and predation rate data may provide additional evidence for the potential cost of gestation under hypoxia in female live-bearing fishes.

CHAPTER 3
AN INTEGRATIVE STUDY OF RESPIRATORY, BEHAVIORAL, AND
REPRODUCTIVE RESPONSES TO CHRONIC HYPOXIA IN THE LIVE-BEARING
SAILFIN MOLLY (*POECILIA LATIPINNA*)

Introduction

Effective uptake of oxygen is critical to the long-term survival of all fishes. The physical properties of water (high viscosity, and density, low concentration of oxygen relative to air) make oxygen uptake a challenge even under normoxic conditions. In addition, there are many systems where water may not remain saturated with oxygen, leading to hypoxia. Hypoxia generally occurs in systems characterized by low light and reduced mixing (Chapter 1; Chapter 5; Carter and Beadle, 1931; Carter, 1934, 1955; Chapman and Liem, 1995; Chapman et al., 1998). Because anthropogenically induced hypoxia is an increasing problem in both freshwater and coastal marine systems (Rosenberg, et al., 1991; Jensen et al., 1993; Chapter 1), an understanding of the effects of hypoxia on fish is of increasing importance.

There are a variety of evolutionary responses by fishes to hypoxia. Air breathing, for example, has evolved many times in euteloests and represents independent specializations in several phylogenetically distinct lineages (Liem, 1980; Kramer, 1987; Graham, 1997). However, the great majority of fishes living hypoxic waters are non-air breathers. Fishes relying on aquatic respiration in hypoxic environments have developed many strategies to increase oxygen transfer from the environment to their tissues and/or

to evade problems associated with hypoxia, include behavioral, morphological, anatomical, physiological, and biochemical adaptations. Some of these responses can be activated over the short-term (e.g., hours, days), while others are genetically fixed and/or developmentally plastic.

Short-term physiological responses, related to biochemical modifications, provide flexibility to deal with variable oxygen habitats and include adjustment of gill perfusion, regulation of the proportions of different hemoglobin (Hb) fractions, adjustment of intraerythrocytic phosphate concentrations or ratios, changes in hematocrit (Hct) and hemoglobin concentration, and metabolic depression (Beamish, 1964; Riggs, 1970; Hughes, 1973; Johansen and Weber, 1976; Johansen et al., 1978; Hughes, 1981; Fievet et al., 1988; Jensen, 1991; Laurent and Perry, 1991; Di Prisco and Tamburini, 1992; Frische and Nilsson, 1993; Jensen, et al., 1993; Perry and McDonald, 1993; Val and de Almeida-Val, 1995).

Adrenergic control of vasoconstriction and dilation at afferent and efferent arterioles of the secondary lamellae also increases recruitment of secondary lamellae of each gill arch (Aota et al., 1990; Kinkead and Perry, 1991). The rate of oxygen uptake can be increased by this elevated perfusion of lamellae (maximizing surface area for exchange) and by increasing ventilation rate (Nilsson, 1986; Frische and Nilsson, 1993; Perry and McDonald, 1993). For example, during exercise or hypoxic stress, a fish may alter patterns of gill blood flow to recruit more lamellae by vasoconstriction or dilation at afferent and efferent arterioles of the secondary lamellae (Frische and Nilsson, 1993; Perry and McDonald, 1993). The rate of oxygen uptake can be quickly increased both by this elevated perfusion of lamellae (maximizing surface area for exchange) and by

increasing ventilation rate (Nilsson, 1986; Frische and Nilsson, 1993; Perry and McDonald, 1993). Blood oxygen transport in most teleosts is dependent on hemoglobin and is normally increased by increasing the number of erythrocytes in circulation, by increasing hemoglobin concentration, and/or by adjusting the affinity of hemoglobin for oxygen. Capacity changes (increased hemoglobin concentration and hematocrit) in response to hypoxia have been reported for several species including rainbow trout (Tetens and Lykkeboe, 1981; Hughes and Kikuchi, 1984; Tun and Houston, 1986; Boutilier et al., 1988; Wells and Weber, 1991); the sheepshead minnow and sailfin molly (Peterson, 1990), some Amazonian fishes (Graham, 1997; Val et al., 1992), Antarctic borch (Wells et al., 1989), and yellowtail (Yamamoto et al., 1985). However, these blood capacity changes are often modest, and some species do not consistently show change (Marinsky et al., 1990). Alterations in blood oxygen affinity are also frequently observed in response to hypoxia and are mediated by decreased erythrocytic concentration of organic phosphates or variation in the ratio of one nucleoside triphosphate compound to another (e.g., GTP/ATP, Bartlett, 1978; Johansen et al., 1978; Weber and Lykkeboe, 1978; Tetens and Lykkeboe, 1981; Val et al., 1992). In addition, teleosts often have complex hemoglobin systems, the components of which sometimes differ markedly in their sensitivity to intraerythrocytic phosphates (Fyhn et al., 1979; Riggs et al., 1979; Perez and Rylander, 1985; Marinsky et al., 1990). To conserve oxygen, circulation to organs not critical to immediate survival needs may also be decreased. Each of these processes can result in a lower aquatic oxygen tension required to maintain an individual's metabolic rate (critical oxygen tension, P_c), and this value is therefore a very useful measure of response to hypoxia.

Despite the diversity of physiological response to hypoxia, several factors restrict these various options. For example, one consequence of increased hematocrit is an increased viscosity of the blood, necessitating an increase in the cardiac work required to pump the blood and the metabolic cost of circulatory work (Houston et al., 1996).

Although an increase in hematocrit would increase blood oxygen carrying capacity, at some point the increased cost that such an increase would put on the circulatory system would outweigh its benefits. In addition, many of the mechanisms described above still do not react rapidly enough to meet oxygen demands when environmental oxygen availability drops at a rapid rate (Hochachka and Somero, 1984; Wootton, 1990).

Behavioral responses provide additional flexibility to deal with rapid variation in dissolved oxygen. Fish may attempt to avoid hypoxic areas through microhabitat selection (Das and Stickle, 1994) or to compensate for low oxygen levels through aquatic surface respiration. (Kramer and Mehegan, 1982). The surface film of water contains high concentrations of oxygen, and many fish can take advantage of this by skimming the surface film and passing this oxygen-rich water over the gills, a behavior referred to as surface skimming or aquatic surface respiration (ASR; Kramer and Mehegan, 1982). Aquatic surface respiration is more efficient than simply increasing ventilation under extreme hypoxia (Kramer and Mehegan, 1982). However, under extreme hypoxia, many fishes spend greater than 90% of their time at the surface (Chapman et al., 1994; Chapman, 1995; Chapman et al., 1995; Olowo and Chapman, 1996). This clearly limits time that can be directed towards foraging, reproduction, and other activities, and may also expose fish to increased risk of aerial predation (Kramer et al., 1983). In addition, many fishes swim continuously at the surface during ASR (ram-assisted ASR, Chapman

et al., 1994, 1995), which may impose a high energetic cost. Thus, one might predict an immediate ASR response that diminishes as physiological compensatory mechanisms become effective, in hours or days of hypoxia exposure. However, little is known about the relationship between behavioral and physiological traits in the acclimation response.

The functional significance of short-term responses to hypoxia exposure is that they allow survival of fishes living in environments that are subject to periodic or chronic hypoxia. Benefits of living in such a harsh environment may include reduced competition for food or shelter or a lower level of predation. However, exposure to hypoxia may also lead to a reduction in fitness-related characters. Studies linking hypoxia exposure to fitness related characters have shown growth depression in long-term studies. For example, Bejda et al. (1992) found that hypoxia limited the growth of young winter flounder, *Pseudopleuronectes americanus*. Similarly, Weber and Kramer (1983) showed that juvenile guppies (*Poecilia reticulata*) sustained high growth rates with access to the water surface, but when access was denied, exhibited a progressive reduction in growth rate with hypoxia. Costs of short-term acclimation effects are less well known but may involve growth depression, negative effects on reproductive effort (e.g. reduced fecundity, smaller propagule size), changes in condition and increased predation rate.

For oviparous fishes, increased costs of parental care (e.g., egg fanning) may limit reproductive success under hypoxia. For live-bearing fishes, costs of hypoxia for gestating females may be particularly high because of evidence that suggests actively developing embryos have a higher mass-specific oxygen requirement than adult fishes. In sailfin mollies, *Poecilia latipinna* (Chapter 2) and several Scorpaenid species of the genus *Sebastes* mass-specific metabolic rates increase in late gestation and possibly limit

reproduction under severe hypoxia (Boehlert and Yoklavich, 1984; Boehlert et al. 1986; Dygert and Gunderson, 1991).

This study examined whether short-term behavioral and physiological responses permit the sailfin molly, *Poecilia latipinna*, the flexibility to deal with extreme hypoxia, and whether there is evidence of an interaction between behavioral (ASR) and physiological responses. The degree to which short-term responses compensate for hypoxia by measuring fitness-related characters (reproduction, fish condition, survivorship) was also addressed. I used an integrative approach combining studies of blood characters, metabolic rates, critical oxygen tension, behavior, reproduction, fish condition, and survival.

Methods

Sailfin mollies used for this study were collected from the backwaters of a salt marsh near Cedar Key, Florida (Chapter 1). Hypoxia was a frequent event at this collection site and was often temporally associated with low tide and isolation from the surrounding system (Chapter 5). Patterns of variation in salinity, dissolved oxygen, and temperature are detailed in Chapter 5.

Hypoxia Acclimation

Experimental Design

Four, six-wk acclimation trials were conducted. The first two trials (1,2) examined behavioral and blood character responses to hypoxia, and the last two trials (3,4) were used to detect changes in metabolic rate and P_c in response to hypoxia. This acclimation length was based on two criteria. First, the acclimation period needed to

encompass the gestation period of females, to ascertain if reproduction was occurring under hypoxia. Second, a time period was selected that might be sufficiently long to detect a tradeoff between physiological and behavioral responses. It was predicted that behavioral responses to hypoxia would change with time, so that differences between normoxic and hypoxic groups would be minimized as physiological compensation proceeded.

Experimental fish were transported to the laboratory in 23 l carboys containing water from the collection site. Once in the laboratory, containers were aerated and allowed to equilibrate with room temperature overnight. The next morning, fish were prophylactically treated with methylene blue for 0.5 h and moved to two 250 l glass aquaria. The brackish water (10 ppt salinity) in each aquarium was a mixture of filtered Atlantic Ocean water (obtained from the C.V. Whitney Laboratory of the University of Florida, Marineland, Florida) and aged tap water. Salinities were checked daily with a Yellow Springs S-C-T Meter and adjusted appropriately. Forty-five fish were introduced into each aquarium at an initial sex ratio of approximately 3 females:1 male. In some cases, fish classified as females were immature males that matured during the course of the 6-wk acclimation period. For the behavioral and hematological analysis (trials 1,2), a subsample of the fish (seven females, five males) was individually marked for identification using tattoo ink (McKinsey and Chapman, 1998; Chapman et al., 1999) prior to introduction into the experimental aquaria. Once the fish were introduced into the aquaria, heaters were used to warm the temperature to 25 °C, and temperature and salinity were monitored daily to maintain these initial levels. For each trial, I used two 250 l aquaria; one was held at normoxia ($7.8\text{--}8.3\text{ mg l}^{-1}\text{ O}_2$) and one was held at extreme

hypoxia ($1.0 \pm 0.10 \text{ mg l}^{-1} \text{ O}_2$) with a microprocessor-based oxygen controller unit utilizing pulses of air or nitrogen to alter aquatic oxygen levels. Developed for this project, this device is based on an MC68HC811E2 microprocessor chip that compares the voltage input from a YSI model 57 oxygen meter to its programmed set points.

Depending on the dissolved oxygen level in the aquarium, the microprocessor opened valves for either nitrogen or air to be bubbled through the water to maintain the desired oxygen level. The dissolved oxygen level used in this study was chosen based on preliminary ASR experiments that showed the 10% ASR threshold (oxygen level at which fish spend an average of 10% of their time engaging in ASR) for *P. latipinna* occurred near $1.0 \text{ mg l}^{-1} \text{ O}_2$. In the hypoxia acclimation, oxygen levels were reduced to 1.0 mg l^{-1} over a 2-day period.

Nitrogenous waste produced by fish in the normoxia aquarium was processed using sponge filters. Because water flow through sponge filters is driven by air bubbling through the filters, this filtration method was not used for the hypoxia aquaria. Sand filtration was chosen for the hypoxia aquarium because biofilter bacteria may be less efficient under low oxygen conditions. The larger surface area of the sand particles makes rapid sand filtration a highly efficient means of removing organic nitrogen from the water by vastly increasing the available surface area for growth of nitrogen-consuming bacteria (Clasen, 1998). Two thirds of the surface area of the hypoxia aquaria was covered by a layer of plastic bubble wrap to minimize oxygen diffusion. The fish were acclimated to a 12L:12D photoperiod and fed Tetra Min ® Flake food *ad libitum* twice daily. Fish were allowed to acclimate to these conditions for seven days prior to beginning experiments. On the 8th day, an aquatic oxygen controller unit was used to

progressively lower the dissolved oxygen level in the hypoxia aquarium to $1.0 \text{ mg l}^{-1} \text{ O}_2$ over three days. Water-quality measures (e.g., nitrogen, ammonia) were checked frequently over the course of the trials and water changes were made as necessary.

For the two acclimation trials (trials 3, 4) used to examine the effect of chronic hypoxia on metabolic rate and critical oxygen tension (P_c), aquaria were set up as described above, but with 13 females and 7 males in each treatment. Table 3-1 summarizes the size characteristics of the groups of fish used in each trial.

Table 3-1. Mean (\pm SE) and range of body mass of sailfin mollies (*Poecilia latipinna*) acclimated to normoxia and hypoxia in four six-wk trials. N represents the sample size remaining at the end of each trial when fish were weighed.

Trial	Treatment	N	Mean Body Mass \pm SE (g)	Range of Body Mass (g)
1	Normoxia	43	2.14 ± 0.08	1.10 – 3.40
	Hypoxia	33	2.00 ± 0.07	1.30 – 3.40
2	Normoxia	36	2.29 ± 0.13	1.10 – 4.10
	Hypoxia	43	2.57 ± 0.17	1.10 – 5.40
3	Normoxia	19	2.78 ± 0.24	1.39 – 5.35
	Hypoxia	19	2.62 ± 0.18	1.16 – 3.98
4	Normoxia	20	2.54 ± 0.20	1.42 – 4.59
	Hypoxia	19	2.41 ± 0.24	1.10 – 4.45

Behavioral Analyses

Behavioral data were recorded on individually marked fish after one, three and five wk of acclimation. The characters selected were traits related to activity and reproductive behaviors. Activity rate was estimated using lines drawn on the aquarium glass at 10-cm intervals. The number of lines crossed by a monitored individual every 10 sec over a 5-min period were recorded. The number of courtship and copulatory encounters every 10 sec over a 5-min observation period were recorded and combined to represent the frequency of reproductive behavior. Whether the monitored individual was the initiate or the recipient of the courtship or copulatory encounters was not differentiated. Similarly, the number of times an individual engaged in ASR in each 10-sec interval over a 5-min period was recorded. Finally, gill ventilation rates were measured as the number of gill ventilations occurring in a 10-sec period. In order to account for temporal differences in behavior, each individual was observed once in the morning (07:30-10:30 h) and once in the afternoon (13:00-16:30 h). It was predicted that swimming activity and reproductive activity would decrease, while ASR and gill ventilation would increase in fishes exposed to hypoxia. Additionally, it was predicted that differences in activity level between normoxic and hypoxic groups would decrease with length of acclimation. Readings were taken a total of six times: morning and afternoon after one, three and five wks of hypoxia acclimation. The average of the two readings for each measured week for each individual was used in behavioral analyses. The acclimation continued for another wk after the last behavioral data were recorded, then hematological sampling was initiated.

Because of strong differences in the behavior of male and female poeciliids reported in other studies (Baird, 1974; Farr, 1989), behavioral data were analyzed separately for males and females. Trial effects were detected by comparing mean frequency of behaviors between trials for each gender and time period using the non-parametric Mann-Whitney U test. Trial effects were evident in only 3% of the analyses, and in those cases, absolute differences were small. Therefore, the two trials were combined to analyze treatment effects. Violation of the homogeneity of variance assumption precluded use of repeated measures ANOVA that included treatment effects. Treatment effects were detected for each time period using the non-parametric Mann-Whitney test (two-tailed significance levels reported).

Blood Analyses

In the first two acclimation trials, fish were sacrificed at the end of the behavioral experiment for hematological sampling. Blood analyses were carried out over a five-day period to minimize any further acclimation effects. Red blood cell count, hematocrit, and hemoglobin content were measured for each individual. Blood was collected by sternal cardiac puncture using two freshly heparinized microhematocrit tubes drawn to a fine point. Once $>20\ \mu\text{l}$ was collected in the first tube, it was immediately dispensed into a heparinized ceramic crucible, and two Eppendorf pipettes then were used to measure aliquots of blood for the red blood cell count and hemoglobin analysis. The second microhematocrit tube was used to determine hematocrit. All blood samples were taken in the afternoon between 1200 and 1800 h. In an attempt to minimize feeding effects during the three-day sampling period, fish remaining in the aquaria were fed only once daily immediately after fish were removed for blood analysis. Therefore, each fish sampled

was nearly 24-h post-absorptive. After blood samples were taken, fish were weighed, measured, and dissected to determine reproductive state. Hemoglobin concentration [Hb] and red blood cell counts (RBC) were determined for both trial replicates. In an effort to maximize available blood samples for [Hb] and RBC in Trial 2, hematocrit was not measured.

Hemoglobin Analysis

Hemoglobin concentration [Hb] was measured spectrophotometrically on 10 μ l samples by conversion to the cyanomethemoglobin derivative (Blaxhall and Daisley, 1973; Innes and Wells, 1985; Murad et al., 1990; Brown, 1993). Cyanomethemoglobin solutions were centrifuged for 10 min at 5000 g to remove erythrocyte debris (Blaxhall, 1972; Blaxhall and Daisley, 1973; Wells et al., 1980; Innes and Wells, 1985). This method has been used reliably by several researchers and is well-suited to small fish because it requires very small quantities of blood (Blaxhall, 1972; Blaxhall and Daisley, 1973; Coburn, 1973; Innes and Wells, 1985).

Erythrocyte Count

Red blood cell counts (RBC) were conducted on a 1:200 dilution of blood in Natt and Herricks solution, a solution that acts as both a stain and dilutant and is routinely used for counting erythrocytes in fish (Cambell and Murru, 1990). Counts were conducted using an improved Neubauer hemacytometer (Brown 1993).

Hematocrit

To determine the packed cell volume of erythrocytes, in 15 min of sampling, 20 μ l microcapillary tubes of blood were sealed and centrifuged (5000 g) for 10 min to separate

plasma from formed elements, such as cell membranes and organelles (Wells et al., 1980; Brown, 1993). A microcapillary reader was used to determine the hematocrit (% erythrocyte).

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated for each individual to estimate the effect of acclimation on the relationship between size of erythrocyte and hemoglobin concentration (Brown, 1993):

$$\text{MCV} = \frac{\text{Hct} \times 10^3}{\text{RBC l}^{-1}}, \quad \text{MCH} = \frac{[\text{Hb}] (\text{g l}^{-1})}{\text{RBC l}^{-1}}, \quad \text{MCHC} = \frac{[\text{Hb}] (\text{g dl}^{-1})}{\text{Hct}}$$

Analysis of variance was used to test for trial, gender, and treatment effects on blood characters. Least-squares means were used to test for differences among means when a fixed effect (e.g., treatment) was significant. Final ANOVA models were determined by sequentially removing non-significant effects. Visual inspection of residual plots and Levene's test for homogeneity of variance indicated that data transformation was unnecessary.

Metabolic Rate and Critical Oxygen Tension

At the end of the metabolic acclimation trials, the metabolic rate and P_c of each fish was estimated using closed respirometry on post-absorptive fish. Metabolic rate was determined for a range of body sizes (Table 3-1). Gestating females were excluded from the analyses due to increased mass-specific RMR in females in late gestation (Chapter 2). Oxygen consumption measurements were performed on unconstrained, post-absorptive fish, that were capable of spontaneous motor activity (Routine Metabolic Rate,

RMR, Winberg, 1956; Fry, 1957). Prior to estimating P_c , total RMR was calculated for each fish using data collected at PO_2 levels above 60 mm Hg at least 30 min after the container was sealed.

Each fish was placed in an opaque Nalgene container (130-210 ml) with an air stone and held for 24 h before the trial at 25 ± 1 °C. At the start of each experiment, the chamber was sealed with an oxygen probe (YSI Model 600). The meter was programmed to measure water temperature and dissolved oxygen concentration at 10-min intervals and to display plotted values throughout the trial. Once the P_c was visually estimated on the computer-generated plots, the experiment was terminated and water in the chamber was quickly returned to normoxia. Following each trial, total length and weight of the fish were recorded. P_c was determined using a BASIC program by Yeager and Ultsch (1989) designed to fit two regression lines to a data set. The critical tension is calculated as the point of intersection of the two lines. Following the six-wk acclimation, RMR and P_c measurements were made over a period of 25 days, and measurements alternated daily between hypoxia-acclimated fish and normoxia-acclimated fish.

Analysis of covariance (ANCOVA) on log-log transformed data, using weight as a covariate, was used to test for effects of body size on P_c for each treatment in each trial. There were no significant body-size effects, and, therefore, analysis of variance (ANOVA) was used to test for trial, gender, and treatment effects on P_c . Least-squares means were used to test for differences among means when a fixed effect (e.g., treatment) was significant. A final ANOVA model was determined by sequentially removing non-significant effects. Visual inspection of residual plots and Levene's test for homogeneity of variance indicated that data transformation was unnecessary. Using weight as a

covariate, ANCOVA was used to test for effects of trial, gender, and treatment on total RMR. Bi-logarithmic transformation was used to linearize RMR data and stabilize variance.

Fitness Correlates

At the end of all four trials, the reproductive state of females was recorded to examine for possible suppression of reproductive activity under extreme hypoxia. In addition, body size (standard length [SL] and total wet weight) of each fish was measured. Reproductive activity was evaluated by scoring the percentage of females with broods, and a chi-squared test of independence was used to test whether the percentage of reproductively active females was independent of treatment in each trial. ANCOVA was used to test for effects of hypoxia on fish weight with standard length as the covariate. Due to differences in the number of fish in each tank between the blood trials and RMR trials, these two sets of data were analyzed separately. Males and females were also analyzed separately, because differential mortality rates between the sexes have been reported under hypoxic conditions (Peterson, 1990). The size range of females in trials one and two were very different (yielding significantly different variances), so the size range where these two trials overlapped was selected for ANCOVA analyses. In each analysis of condition, weight and length were log transformed to produce linear relationships.

Results

Behavior

Females used for the behavior analyses averaged 2.13 g in body mass (range=1.8 to 3.6 g), and males averaged 2.3 g (range=1.7 to 3.4 g). There was no treatment effect on activity in either male or female mollies in any of the three time periods (Mann-Whitney tests, $P>0.05$, Table 3-2). However, treatment effects were observed for all other behavioral categories. Aquatic surface respiration was not observed in normoxia-acclimated sailfin mollies, but did occur in hypoxia-acclimated individuals. In females, ASR frequency was highest at wk 1 and wk 3 for fish acclimated to hypoxia, but by wk 5, ASR behavior was very infrequent, and there was no detectable difference in ASR frequency between hypoxia- and normoxia-acclimated individuals (Figure 3-1, Table 3-2). For males, ASR frequency was higher in the hypoxia treatment at wk 3, but ASR behavior became very infrequent by the second sampling period, and there was no difference in ASR frequency between hypoxia and normoxia-acclimated individuals at wk 3 or wk 5 (Figure 3-1, Table 3-2). Gill ventilations in both female and male mollies were much higher in the hypoxia treatment throughout the acclimation period (Table 3-2), and did not decline with exposure time. For males, the frequency of reproductive behaviors was lower in hypoxia-acclimated fish than in normoxia-acclimated fish during the first two sampling periods; however, by the end of the acclimation trial, there was no significant treatment effect (Table 3-2). For females, there was no difference in the frequency of reproductive behaviors in any of the three sampling periods, nor did reproductive behavior change over time (Table 3-2).

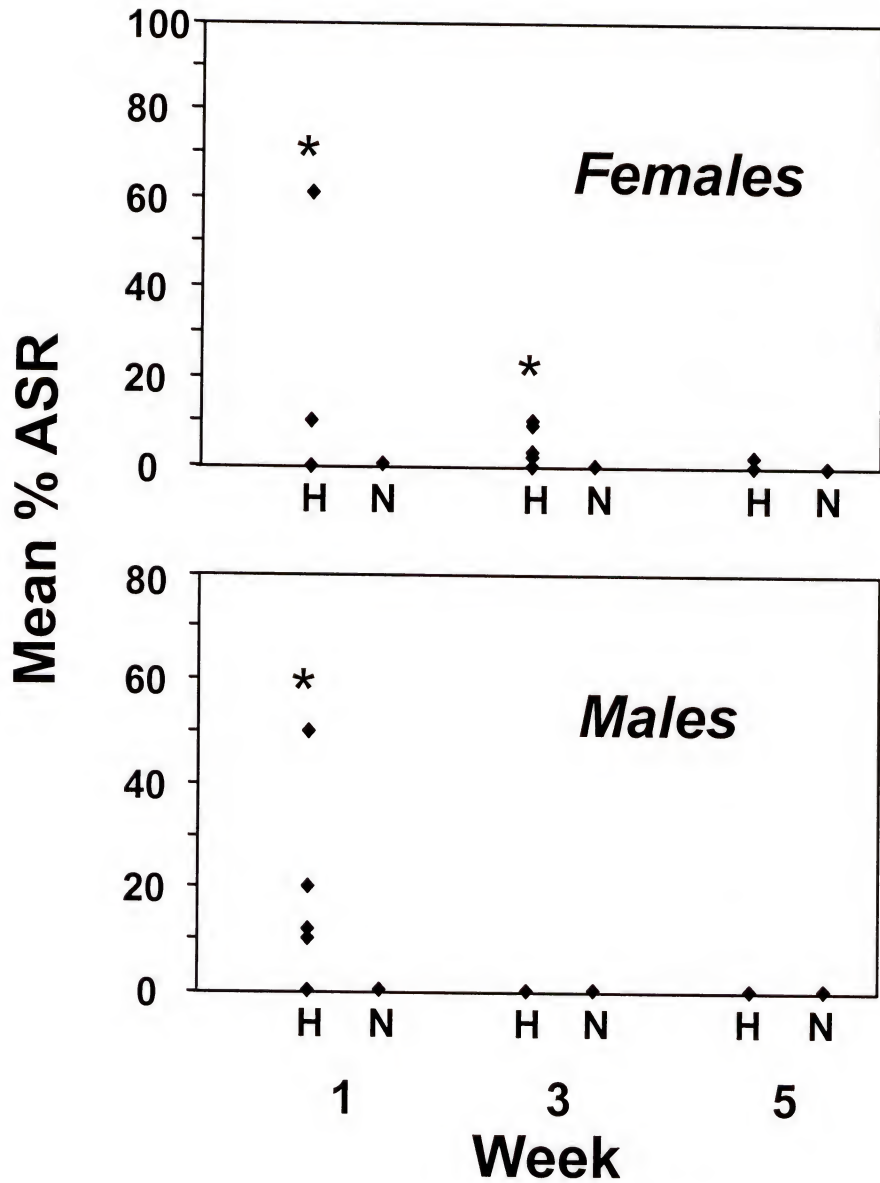


Figure 3-1. The effect of acclimation (normoxia, $N=7.8-8.3 \text{ mg l}^{-1} \text{ O}_2$; hypoxia, $H=1.0 \pm 0.10 \text{ mg l}^{-1} \text{ O}_2$) on mean % time spent conducting ASR for 10 male and 14 female *Poecilia latipinna* over a six wk acclimation period. Diamonds are mean % ASR of individuals (see Table 3-1 and text for sample sizes). Significant acclimation effects (*= $p<0.05$) disappeared by wk 3 for males and by wk 5 for females.

Table 3-2. Summary of behavioral data for 14 female and 10 male sailfin mollies (*Poecilia latipinna*) acclimated for six wk to normoxia (7.8-8.3 mg l⁻¹ O₂) or extreme hypoxia (1.0 ± 0.10 mg l⁻¹ O₂). The median and range are presented for each character and the P-value representing the probability of a significant difference between acclimation treatments (Mann-Whitney U tests). NS: P>0.05. See methods for explanation of behavioral categories.

Behavior	Gender	Week	Normoxia		Hypoxia		P-value
			Median	Range	Median	Range	
Activity (# lines Crossed 10 sec ⁻¹)	Male	1	13.7	11.7 – 20.4	13.7	11.7 – 20.4	NS
		3	13.8	10.3 – 18.7	16.4	11.9 – 20.6	NS
		5	15.3	11.2 – 24.4	14.6	12.6 – 21.1	NS
	Female	1	15.7	14.1 – 23.5	15.3	13.1 – 20.4	NS
		3	13.7	11.2 – 22.2	14.5	12.2 – 21.2	NS
		5	17.8	3.2 – 22.4	15.6	9.8-21.7	NS
ASR (frequency 10 sec ⁻¹)	Male	1	0	0 – 0	0	0 – 0.5	0.031
		3	0	0 – 0	0	0 – 0	NS
		5	0	0 – 0	0	0 – 0	NS
	Female	1	0	0 – 0	0	0 – 0.6	0.011
		3	0	0 – 0	0	0 – 0.1	0.011
		5	0	0 – 0	0	0 – 0.02	NS
Reproduction (frequency 10 sec ⁻¹)	Male	1	0.8	0 – 1.8	0	0 – 0.2	0.010
		3	2.2	0 – 4.8	0	0 – 2.1	0.005
		5	0.1	0 – 1.4	0.1	1 – 1.1	NS
	Female	1	0.1	0 – 0.3	0	0 – 0.1	NS
		3	0.1	0 – 0.6	0	0 – 1.1	NS
		5	0.1	0 – 0.4	0	0 – 0.4	NS
Ventilation (# gill vents 10 sec ⁻¹)	Male	1	12.0	11.2 – 15.2	25.9	23.7 – 27.5	<0.001
		3	12.1	10.6 – 14.9	27.0	23.7 – 29.2	<0.001
		5	12.0	11.2 – 16.1	28.7	23.7 – 33.1	<0.001
	Female	1	12.2	11.2 – 17.8	28.2	25.4 – 33.1	<0.001
		3	11.8	10.6 – 18.4	28.7	25.2 – 36.1	<0.001
		5	12.1	10.1 – 16.0	29.7	28.0 – 36.1	<0.001

Blood Analysis

Fish acclimated to hypoxia appeared to compensate by increasing the number of RBC and [Hb] of the blood (Table 3-3, Figure 3-2). There were no significant effects of trial or gender on [Hb] or RBC, nor were any interaction terms significant (Table 3-3). Thus, the final ANOVA models included only treatment effects ([Hb]: $F=27.14$, $p<0.001$; RBC: $F=4.81$, $p=0.03$). Sailfin mollies acclimated to hypoxia showed higher [Hb] and higher RBC than fish acclimated to normoxia (Figure 3-2). In the full ANOVA model, there were no significant effects of gender, treatment, or their interaction on hematocrit. However, removal of gender and the interaction term from the model indicated that hematocrit was marginally higher ($F=3.57$, $p=0.064$, Figure 3-2) in the hypoxia treatment. Because there were no trial or gender effects on the hematological characters, only treatment effects were tested for the integrative parameters: MCH, MCHC and MCV. MCH was higher in fish acclimated to hypoxia ($n=112$; normoxia: mean= 20.72 ± 1.52 (SE); hypoxia: mean= 25.28 ± 1.32 , $t=-2.24$, $p=0.027$). However, MCHC did not differ between treatments ($n=49$; normoxia: mean= 23.11 ± 2.08 (SE); hypoxia: mean= 24.54 ± 2.40 (SE); $t=-0.450$, $p=0.655$) nor did MCV ($n=56$; normoxia mean= 12.50 ± 1.81 ; hypoxia: mean= 10.93 ± 1.54 , $t=0.656$, $p=0.514$).

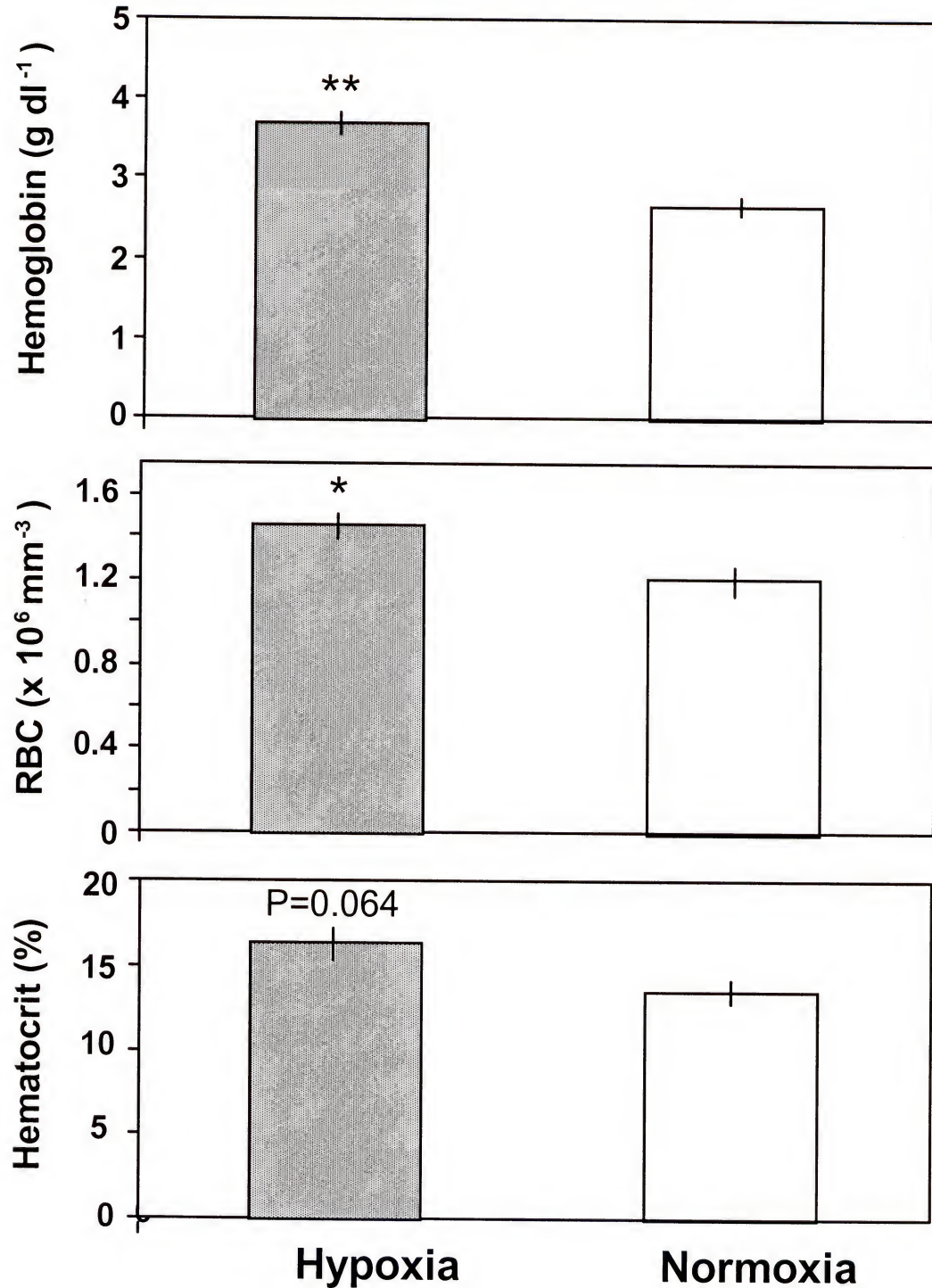


Figure 3-2. Mean hemoglobin concentration [Hb] (g dl⁻¹) and red blood cell count (RBC x 10⁶mm⁻³) for sailfin mollies (*Poecilia latipinna*) acclimated for six wk to normoxia (7.8-8.3 mg l⁻¹ O₂) or extreme hypoxia (1.0 \pm 0.10 mg l⁻¹ O₂). Vertical lines represent \pm SE. *= p <0.05, **= p <0.001.

Table 3-3. Effects of trial, acclimation treatment (normoxia vs hypoxia), gender, and their interaction on three hematological characters: hemoglobin concentration (g dl^{-1}), red blood cell count, and hematocrit (%) in sailfin mollies (*Poecilia latipinna*).

Hemoglobin concentration

Source of Variation	df	MS	F	p-value
Trial	7	1.98	1.83	0.18
Gender	1	0.61	0.56	0.454
Dissolved oxygen	1	22.71	20.92	<0.001*
T X G	1	0.49	0.45	0.504
T X DO	1	1.37	1.27	0.263
G X DO	1	0.000014	0.00	0.997
T X G X DO	1	0.68	0.62	0.432
Error	111	1.09		

Red blood cell count

Source of Variation	df	MS	F	p-value
Trial	7	0.0032	0.08	0.778
Gender	1	0.18	0.44	0.511
Dissolved oxygen	1	1.87	4.57	0.035*
T X G	1	0.083	0.20	0.654
T X DO	1	0.18	0.43	0.512
G X DO	1	0.18	0.44	0.510
T X G X DO	1	0.18	0.45	0.503
Error	114	0.41		

Hematocrit

Source of Variation	df	MS	F	p-value
Gender	1	12.21	0.41	0.524
Dissolved oxygen	1	81.19	2.74	0.104
G X DO	1	1.66	0.056	0.814
Error	50	29.65		

Critical Oxygen Tension and Routine Metabolic Rate

Fish acclimated to hypoxia showed a lower P_c , but effects on RMR differed between trials. For P_c , significant trial and treatment effects were noted, but there were no significant interaction terms indicating that treatment effects were similar between the two trials (Table 3-4). The final model included treatment ($F=23.61$, $p<0.001$) and trial ($F=6.01$, $p=0.017$) as the only independent effects. In both trials, P_c was lower in hypoxia-acclimated fish (Figure 3-3).

Table 3-4. Results of ANOVA showing the effects of trial, gender, and acclimation to six wk of hypoxia ($1.0 \pm 0.10 \text{ mg l}^{-1} \text{ O}_2$) or normoxia ($7.8\text{-}8.3 \text{ mg l}^{-1} \text{ O}_2$) and their interaction on the critical oxygen tension of sailfin mollies (*Poecilia latipinna*).

Critical oxygen tension

Source of Variation	df	MS	F	p-value
Trial	7	0.030	5.39	0.024*
Gender	1	0.0014	0.25	0.620
Dissolved oxygen	1	0.105	19.17	<0.001*
T X G	1	0.000054	0.01	0.921
T X DO	1	0.0012	0.22	0.642
G X DO	1	0.000014	0.00	0.997
T X G X DO	1	0.0035	0.63	0.430
Error	50	0.0055		

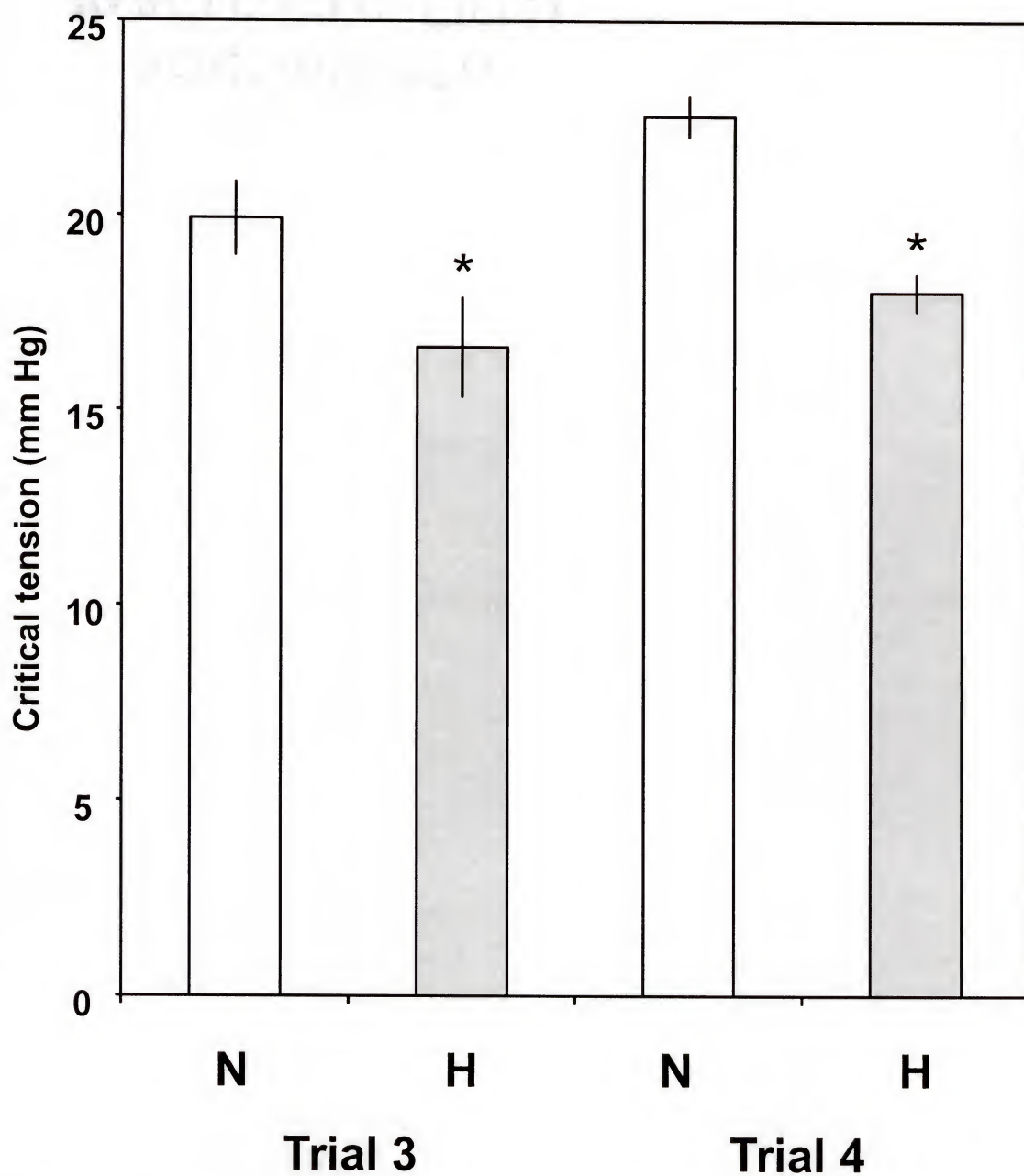


Figure 3-3. Mean critical oxygen tension for sailfin mollies (*Poecilia latipinna*) acclimated for 6 wk to extreme hypoxia ($H=1.0 \pm 0.10 \text{ mg l}^{-1} \text{ O}_2$) or normoxia ($N=7.8\text{--}8.3 \text{ mg l}^{-1} \text{ O}_2$). Trials are presented separately due to significant trial effects. Vertical lines represent $\pm \text{SE}$. * $=p<0.05$.

ANCOVA indicated differences in the slopes of the mass-RMR relationship between trials, so trials were treated separately for further analysis of total RMR. In both trials body size was a highly significant covariate (Table 3-5) and gender effects were not significant. In Trial 3, RMR was depressed under hypoxia. There was no treatment effect in Trial 4 (Table 3-5).

Table 3-5. ANCOVA analyses showing the effects of acclimation to 6 wk of normoxia ($7.8\text{--}8.3\text{ mg l}^{-1}\text{ O}_2$) or extreme hypoxia ($1.0 \pm 0.10\text{ mg l}^{-1}\text{ O}_2$), gender, and their interaction on the intercepts of the relationship between log metabolic rate (RMR) and log weight in sailfin mollies (*Poecilia latipinna*). Adjusted means (sample means adjusted for a common mean body mass and a common regression line) were calculated from the ANCOVA analysis.

Metabolic rate- Trial 3

Source of Variation	df	MS	F	p-value	Adjusted means	
					Hypoxia RMR mg O ₂ hr ⁻¹	Normoxia RMR mg O ₂ hr ⁻¹
Log weight	1	0.16	29.58	<0.001*	0.47±1.04	0.55±1.05
Gender (G)	1	0.0041	0.74	0.396		
Dissolved Oxygen (DO)	1	0.0041	7.40	0.011*		
G X DO	1	0.014	2.60	0.120		
Error	29	0.0055				

** mean body mass=2.5 g

Metabolic rate- Trial 4

Source of Variation	df	MS	F	p-value	Adjusted means	
					Hypoxia VO ₂ mg O ₂ hr ⁻¹	Normoxia VO ₂ mg O ₂ hr ⁻¹
Log weight	1	1.01	153.05	<0.001*	0.38±1.05	0.39±1.05
Gender (G)	1	0.0015	2.23	0.144		
Dissolved Oxygen (DO)	1	0.0018	0.27	0.610		
G X DO	1	0.0011	1.61	0.213		
Error	29	0.0055				

**mean body mass=2.3 g

Fitness Correlates

Analyses of covariance of weight-length regressions for hypoxia-acclimated and normoxia-acclimated fish indicated no significant differences in the slopes of weight on length between treatment groups of males and females (Table 3-6). However, there were significant differences in the Y-intercepts for males of all trials, suggesting that hypoxia-acclimation resulted in a significant loss of condition in males (Table 3-6). Chi-squared analyses indicated no increase in mortality associated with chronic hypoxia acclimation for either gender (Table 3-7). The percentage of reproductively active females (females with eggs or embryos at the end of the trial) did not differ between treatments in any of the four trials (chi-squared tests, $P > 0.05$, Table 3-8) indicating no significant depression of reproductive activity in response to hypoxia acclimation.

Table 3-6. The results of ANCOVA analyses showing the effects of acclimation to 6 wk of normoxia ($7.8\text{--}8.3\text{ mg l}^{-1}\text{ O}_2$) or extreme hypoxia ($1.0 \pm 0.10\text{ mg l}^{-1}\text{ O}_2$) on the bilogarithmic relationship between standard weight and length for male and female *Poecilia latipinna*. Adjusted means (sample means adjusted for a common mean body mass and a common regression line) were calculated from the ANCOVA analysis.

Trial	Gender	Treatment	N	Slope		Intercept		Adjusted Mean \pm SE (g)
				F	P	F	P	
1 and 2	Male	Normoxia	25	3.022	0.089	8.538	0.005*	2.48 ± 0.06
		Hypoxia	29					2.25 ± 0.01
	Female	Normoxia	21	4.066	0.051	0.865	0.179	2.59 ± 0.07
		Hypoxia	26					2.65 ± 0.07
3 and 4	Male	Normoxia	13	0.458	0.507	3.769	0.033*	2.67 ± 0.17
		Hypoxia	12					2.24 ± 0.15
	Female	Normoxia	26	2.439	0.125	2.393	0.065	2.55 ± 0.01
		Hypoxia	26					2.33 ± 0.01

Table 3-7. Percent of reproductively (reprod.) active female sailfin mollies (*Poecilia latipinna*) after 6 wk acclimation to hypoxia ($1.0 \pm 0.10 \text{ mg l}^{-1} \text{ O}_2$) or normoxia ($7.8\text{-}8.3 \text{ mg l}^{-1} \text{ O}_2$). Results are presented separately for the four 6-wk trials.

Trial	Treatment	N	Percent Non- Reprod.	Percent Reprod.	Percent With Egg	Percent With Embryo	chi- square	df	p-value
1	Normoxia	25	64.0	36.0	24.0	16.0	0.37	1	0.424
	Hypoxia	18	61.1	38.9	22.2	16.7			
2	Normoxia	23	87.0	13.0	0.0	13.0	0.225	1	0.318
	Hypoxia	32	87.5	12.5	12.5	0.0			
3	Normoxia	13	61.5	38.5	7.7	30.8	0.022	1	0.44
	Hypoxia	13	61.5	38.5	30.8	7.7			
4	Normoxia	13	92.3	7.7	0.0	7.7	0.000	1	1.000
	Hypoxia	13	92.3	7.7	7.7	0.0			

Table 3-8. The results of chi-square analyses showing the effects of acclimation to 6 wk to normoxia ($7.8\text{--}8.3\text{ mg l}^{-1}\text{ O}_2$) or extreme hypoxia ($1.0 \pm 0.10\text{ mg l}^{-1}\text{ O}_2$) on survival in *Poecilia latipinna*.

Trial	Gender	Treatment	N	Percent	chi-square	df	p-value
				Survival			
1 and 2	Male	Normoxia	24	62.5	0.107	1	0.372
		Hypoxia	24	83.3			
	Female	Normoxia	66	87.9	0.023	1	0.439
		Hypoxia	66	89.4			
3 and 4	Male	Normoxia	13	92.9	0.037	1	0.500
		Hypoxia	12	85.7			
	Female	Normoxia	26	100	0.000	1	1.000
		Hypoxia	26	100			

Discussion

In fishes, plastic behavioral and physiological responses to hypoxia may be critical for species living in habitats characterized by strong spatial and temporal variation in dissolved oxygen content. The results of this study demonstrate physiological and behavioral responses in *P. latipinna* held at $1.0 \text{ mg l}^{-1} \text{ O}_2$ for a six-wk acclimation period that decreased their P_c and increased their oxygen uptake capacity. The use of ASR was an immediate response to hypoxia that declined over the acclimation period. This suggests a more gradual physiological compensation that decreased the need for ASR. Behavioral and physiological mechanisms in this species seem to provide sufficient compensation to permit reproduction to continue under extreme hypoxia and to mitigate mortality. However, there may be predation costs in the field related to time allocated to ASR.

Many biochemical and physiological mechanisms that enhance oxygen uptake capacity seem to have a longer response time than adaptive behavioral responses (Hochachka and Somero, 1984; Wootton, 1990). The results of this study support the concept of a tradeoff between behavioral and physiological response in the hypoxia treatment. Although hematological response was only recorded at the end of the acclimation trial, other studies have shown physiological changes that increase oxygen transfer occurring over a much shorter time frame. The cumulative effects of such changes may permit a decrease in the threshold for ASR. These include: increased gill ventilation rate (this study; Frische and Nilsson, 1993); cardio-respiratory synchronization, as in carp (Glass et al., 1991); catecholamine-mediated adjustments such

as the release of stored erythrocytes from the spleen or liver (Perry and McDonald, 1993), as in the blind cave cyprinid *Phreatichthys andruzzii* (Frangioni et al., 1997); increased hemoglobin binding affinity through a reduction in intraerythrocytic nucleotide triphosphates (NTP), as in carp, goldfish, and trout (Weber et al., 1976a,b; Weber and Lykkeboe, 1978; Greaney and Powers, 1978; Murad et al., 1990); and regulation of the pH environment of hemoglobin (Nikinmaa, 1983).

Behavior

The decrease in ASR frequency with acclimation time observed in this study suggests that there are costs to the behavioral response that favor physiological compensation. Poeciliids appear generally well-suited to survive hypoxic conditions, having a dorsally flattened head and up-turned lips that allow them to skim the oxygen rich water surface layer while maintaining a horizontal swimming position (McKinsey and Chapman, 1998). This suggests that their primary coping mechanism for hypoxia would be ASR. Other, short-term studies have shown that without access to the surface, mollies sustain high mortality rates at low oxygen levels comparable to those used in this experiment (Peterson, 1990). However, in this study, the frequency of ASR in the hypoxia acclimation treatment was low and declined in both sexes over the acclimation period, more quickly in males than females, and was nearly absent by the end of the experiment. Perhaps access to ASR is critical to survival during the early stages of hypoxia, while individuals physiologically adjust their blood oxygen affinity and capacity to deal with low oxygen stress. ASR is a widespread behavioral response to extreme aquatic hypoxia in fishes (Gee et al., 1978; Kramer and McClure, 1982; Saint-Paul and Soares, 1987; Chapman et al., 1994, 1995; Val and de Almeida-Val, 1995; Olowo and

Chapman, 1996). However, ASR has high energetic costs and leads to an increased risk of aerial predation (Kramer et al., 1983). The population used for this study is found in a highly variable, estuarine environment where hypoxia and aerial predation are not uncommon threats (Chapter 4). Therefore, their minimal dependence on ASR to cope with extreme chronic hypoxia should not be surprising. When a female was found frequently at the surface engaging in ASR, it was often an indication of impending parturition. Females in late gestation more frequently engage in ASR, which may reflect their increasing brood oxygen demands (Chapter 2).

Ventilation rate was much higher for fish held under hypoxic than normoxic conditions, but showed no detectable change with acclimation duration. It is likely that other physiological mechanisms (e.g., increased [Hb], RBC) are not sufficient to permit a reduction in ventilation rate over time. It is also possible that gill ventilation volume decreased over the acclimation period, a character that was not measured in this study. Similar increases in ventilation rate in response to hypoxia have been previously documented in *P. latipinna* and in numerous other species (Peterson, 1990; Lomholt and Johansen, 1979; Boese, 1988; Chapman et al., 1994, 1995; Olowo and Chapman, 1996; Chapman and Chapman, 1998), as have increases in ventilation volume (Holeton and Randall, 1967; Smith and Jones, 1982; Randall, 1990). Increased ventilation minimizes hypoxia stress both by increasing oxygen uptake and enhancing convective conditions for CO₂ removal (Brauner and Randall, 1998). However, Peterson (1990) suggested that increased plasma osmolality found during hypoxic exposure could be a result of increased ventilation rates. Thus, as a by-product of maximizing oxygen uptake at the gills (through increased blood perfusion of the gill lamellae and increased ventilation rates),

there may be increased exposure of the blood to osmotic pressures of the aquatic environment. For example, in hypoxia, a fish in sea water may tend to lose more water to the salty environment, increasing plasma osmolality. In this experiment, 10 ppt salinity was selected to minimize osmoregulatory stress (Evans, 1975; Nordlie et al., 1992).

Metabolic Rate and Critical Oxygen Tension

Physiological characters also indicated high tolerance to hypoxia in sailfin mollies. Individuals from the population of mollies studied had lower P_c values when acclimated to hypoxia than normoxia. Their increase in blood oxygen carrying capacity in response to hypoxia acclimation probably contributed to the decrease in P_c . The increase in blood oxygen carrying capacity discussed above probably contributed to the decrease in P_c measured in hypoxia-acclimated individuals. A reduction in P_c in response to chronic hypoxia acclimation has been found in other species as well (Table 3-10). Relative to several other freshwater fishes, sailfin mollies show an extremely low P_c , both for normoxia- and for hypoxia-acclimated individuals. However, the values fall in the range of swamp-dwelling fishes representing a phylogenetically diverse suite of taxa (Table 3-9).

Table 3-9. Comparison of critical oxygen tensions (P_c) of fish of various habitats and activity levels.

Species	°C	P_c (mm Hg)	Description	Reference
<i>Temperate Well-oxygenated Habitat</i>				
<i>Salvelinus fontinalis</i> Salmonidae	15	85	Cold water streams, North American	Beamish, 1964
<i>Oncorhynchus mykiss</i> Salmonidae:	12	90	Cold water streams, North America	Marvin and Heath, 1968
<i>Lepomis macrochirus</i> Centrarchidae	20	90	Lakes and rivers, North America	Burton and Heath, 1980
<i>Micropterus salmoides</i> Salmonidae	20	40	Lakes and rivers, North America	Cech et al., 1979
<i>Etheostoma rufilineatum</i> Percidae	20	105	Fast flowing rivers and streams, North America	Ultsch et al., 1978
<i>Temperate Periodically Hypoxic Habitat</i>				
<i>Cyprinodon variegatus</i> Cyprinodontidae	20	54	Estuarine population, North America	Haney, 1995
<i>Etheostoma boschungii</i> Percidae	20	30	Slack water habitats of streams, North America	Ultsch et al., 1978

Table 3-9--continued

Species	°C	P _c (mm Hg)	Description	Reference
<i>Tropical Well-oxygenated Habitat</i>				
<i>Amphilius jacksonii</i> Amphiliidae	25	27	Dura River, Africa	Walsh et al., unpubl. data
<i>Hoplias lacerdae</i> Erythrinidae	25	35	Well-aerated streams, South America	Rantin et al., 1992
<i>Astatotilapia burtoni</i> Cichidae	25	25	Lake Tanganyika, Africa	Verheyen et al., 1994
<i>Dimidiochromis compressiceps</i> Cichidae	25	36	Lake Malawi, Africa	Verheyen et al., 1994
<i>Melanchromis auratus</i> Cichidae	25	25	Lake Malawi, Africa	Verheyen et al., 1994
<i>Oreochromis niloticus</i> Cichlidae	25	31	Lake Victoria, Africa	Verheyen et al., 1994
<i>Tropheus moorii</i> Cichidae	25	47	Lake Tanganyika, Africa	Verheyen et al., 1994
<i>Lates niloticus</i> Centropomidae	25	24	Lake Nabugabo, Africa	Schofield and Chapman, 2000
<i>Prochilodus scrofa</i> Prochilodontidae	25	22	South America	Fernandes et al., 1995

Table 3-9--continued

Species	°C	P _c (mm Hg)	Description	Reference
<i>Tropical Periodically Hypoxic Habitat</i>				
<i>Petrocephalus catostoma</i> Mormyridae	25	9	Lwamunda swamp, Africa	Chapman and Chapman, 1998
<i>Barbus neumayeri</i> Cyprinidae	25	9	Rwembaita swamp, Africa	Chapman and Chapman, unpubl. Data
<i>Hoplias malabaricus</i> Erythrinidae	25	20	Stagnant waters, South America	Rantin et al., 1992
<i>Astatotilapia velifer</i> Cichlidae	25	13	Lake Nabugabo, Africa	Rosenberger and Chapman, 2000
<i>Prognathochromis venator</i> Cichlidae	25	13	Lake Kayanja, Africa	Rosenberger and Chapman, 2000
<i>Pseudocrenilabrus multicolor victoriae</i> Cichidae	25	7	Lake Nabugabo, Africa	Rosenberger and Chapman, 2000
<i>Experimental Acclimation to Chronic Hypoxia</i>				
<i>Carassius auratus</i> Cyprinidae	23	40 25	Normoxia Hypoxia 28 d	Lumholt and Johansen, 1979
<i>Poecilia latipinna</i> Poeciliidae	25	23 19	Normoxia Hypoxia 42 d	This study

In general, the metabolic rate of *P. latipinna*, both normoxia- and hypoxia-acclimated fish, was low falling well below the standard curve for fishes developed by Winberg (1961) and also below Winberg's standard curve for cyprinodontiforms (Figure 3-4, Winberg, 1961). This indicates a low metabolic rate relative to other closely related species. A low routine metabolic rate decreases oxygen demand and may be a useful strategy in hypoxic environments. Studies of swamp-dwelling fishes in Africa have noted low routine metabolic rates in several species (Chapman and Chapman 1998, Rosenberger and Chapman 2000, Chapman and Chapman unpubl. data). Although both trials were treated equally, it is possible that the minor metabolic depression occurring in Trial 3 could be attributed to either variations in spontaneous motor activity during oxygen consumption measurements, or to seasonal acclimatization effects. Such seasonal effects may be associated with reproductive activity levels, which also differed significantly between trials. Metabolic depression, correlated with lower levels of spontaneous motor activity, was exhibited after acclimation to 4 wk in chronic hypoxia (≈ 30 mm Hg), in *Cyprinus carpio* (Lumholt and Johansen, 1979).

Petersen (1990) measured RMR of sailfin mollies under hypoxic conditions and reported an 83% drop in RMR at $PO_2 = 40$ mm Hg. The results of the Peterson study are not directly comparable to the current study, because RMR in this study were only measured under normoxic conditions. In addition, in Petersen's study, metabolic rates were measured after only 2.5 h of acclimation to hypoxia. It is possible that the longer acclimation period in the Peterson study would have permitted a greater degree of hematological compensation and minimized the degree of metabolic depression. For example, common carp, *Cyprinus carpio* acclimated to chronic hypoxia (≈ 30 mmHg)

maintain 30-40% higher RMR under hypoxic conditions than normoxia acclimated (>120 mm Hg) individuals (Lumholt and Johansen, 1979). Additionally, like sailfin mollies, carp decrease their critical tensions (P_c) in response to acclimation to chronic hypoxia (P_c Normoxia \cong 40 mm Hg; P_c Hypoxia \cong 25 mm Hg; Lumholt and Johansen, 1979). The erythrinid, *Hoplias malabaricus*, (an exclusive water-breather) reacted similarly to acclimation to chronic hypoxia (two and four wk at 25 mm Hg). Acclimation to over two wk of hypoxia allowed *Hoplias malabaricus* to maintain significantly higher metabolic rates under hypoxic conditions (15 mm Hg), and to reduce its P_c (P_c Normoxia \cong 50 mm Hg; P_c Hypoxia \cong 25 mm Hg; Rantin and Johansen, 1984).

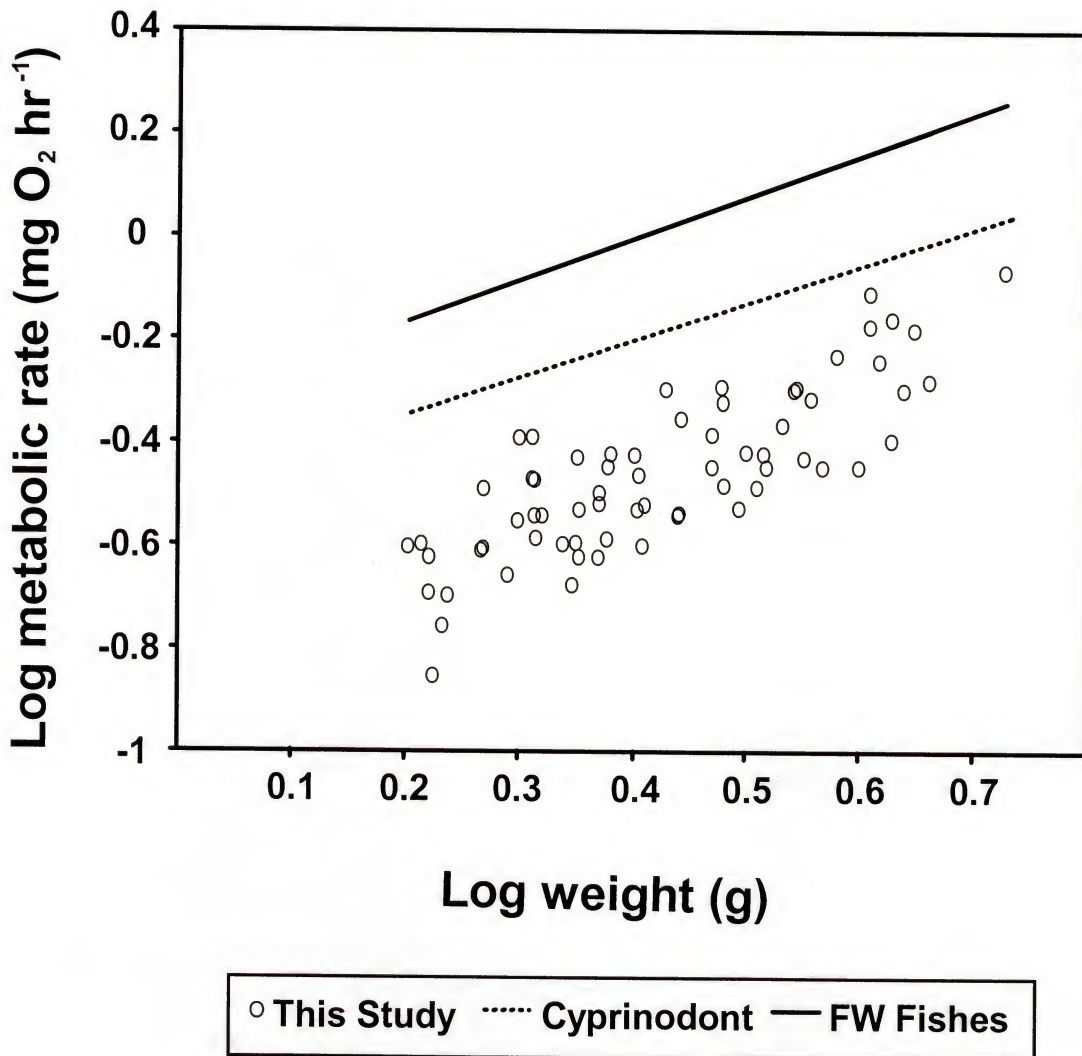


Figure 3-4. The relationship between \log_{10} weight and \log_{10} metabolic rate ($\text{mg O}_2 \text{ hr}^{-1}$) for *Poecilia latipinna*. These data represent the combined results of Trials 3 and 4. The standard curve for freshwater (FW) fishes and the standard curve for cyprinodontiforms developed by Winberg (1961) are presented for comparison.

Hematological Characters

Poecilia latipinna appears to increase blood oxygen capacity primarily through increases in circulating red blood cells and blood hemoglobin concentration. Increases in red blood cell count, hematocrit, and/or hemoglobin concentration with hypoxia acclimation have also been reported in several species representing a phylogenetically diverse suite of taxa and ecological groupings (Table 3-10). Examples include the highly active rainbow trout *Oncorhynchus mykiss* (Boutilier et al., 1988); the facultative air-breathers *Pterygoplichthys multiradiatus* (Val et al., 1990) and *Hoplosternum thoracatum* (Val et al., 1988), and the estuarine *Cyprinodon variegatus* (Peterson, 1990). However, Murad et al. (1990) noted that while many species have been reported to increase circulating erythrocytes in response to extreme hypoxia, these increases are not consistent in their magnitude. Furthermore, in some species, no change in Hct has been reported (Tetens and Lykkeboe, 1981; Murad et al., 1990; Wells and Weber, 1990; Table 3-7).

Table 3-10. Comparative data on the red blood cell count (RBC), hematocrit (Hct), and hemoglobin concentration [Hb] values for a suite of teleost species for both normoxic ($> 7 \text{ mg l}^{-1} \text{ O}_2$) and hypoxic ($< 3 \text{ mg l}^{-1} \text{ O}_2$) conditions. (*) indicates that the species is a facultative air breather. "Field" indicates measurements taken on individuals collected from the field. Experimental acclimation duration is indicated in h, d or wks.

Species	RBC X 10^6 mm^{-3}	Hct %	[Hb] (g dl^{-1})	Conditions	Reference
<i>Carassius auratus</i>		22.5	5.6	Normoxia	Murad et al., 1990
Cyprinidae		25.4	6.3	Hypoxia-3 h	
<i>Cyprinus carpio</i>	0.95	26.4	4.4	Normoxia	Weber and Lykkeboe, 1978
Cyprinidae	1.02	29	4	Hypoxia-30 d	
<i>Colossoma</i>		22.7	6.9	Normoxia	Val et al., 1992
<i>macropomum</i> *		28.5	8.5	Hypoxia-30 d	
Characidae					
<i>Piabucina festae</i> *		44	12.7	Wet season-field	Graham, 1985
Characidae		46.6	14.6	Hypoxia-14 to 21d	
<i>Piaractus</i>		27.3	8.2	Normoxia	Val et al., 1992
<i>brachypomum</i> *		31.2	8.6	Hypoxia-30 d	
Serrasalminidae					
<i>Hypostomus</i>		24.9	5.1	Wet season-field	Graham, 1985
<i>plecostomus</i> *		32.8	7.9	Hypoxia-14 to 21d	
Loricariidae					
<i>Loricaria uracantha</i> *		22	4.9	Wet season-field	Graham, 1985
Loricariidae		23.5	4.6	Hypoxia-14 to 21d	
<i>Pterygoplichthys</i>	1.5	29.8	9.22	Normoxia	Val et al., 1990
<i>multiradiatus</i> *	1.6	30.4	11.35	Hypoxia-30 d	
Loricariidae					
<i>Ancistrus chagresi</i> *		23.5	4.6	Wet season-field	Graham, 1985
Loricariidae		31.2	6.4	Hypoxia-14 to 21d	
<i>Ictalurus punctatus</i>		34.6	7.74	Normoxia	Scott and Rogers, 1981
Ictaluridae		32.6	10.42	Hypoxia 72 h	
<i>Oncorhynchus mykiss</i>		28.2	7.95	Normoxia	Tetens and Lykkeboe, 1981;
Salmonidae		35.3	7.15	Hypoxia	

Table 3-10-- continued.

Species	RBC X 10 ⁶ mm ⁻³	Hct %	[Hb] (g dl ⁻¹)	Conditions	Reference
<i>Oncorhynchus mykiss</i>		25.1		Normoxia	Wells and Weber, 1990
Salmonidae		19.2		Hypoxia 14 d	
		32.8		Hypoxia 1h	
<i>Hoplosternum littorale</i> *		39.1	12.9	Normoxia	Val et al., 1992
Callichthyidae		43.7	16.1	Hypoxia-30 d	
<i>Hoplosternum thoracatum</i> *		35.9	9.3	Wet season-field	Graham, 1985
Callichthyidae		38.9	9.2	Hypoxia-14 to 21d	
<i>Gadus morhua</i>		26	5.3	Normoxia	Scott and Rogers, 1981
Gadidae		23	5.5	Hypoxia-6 h	
<i>Pomatoschistus minutus</i>			3.53	Normoxia	Petersen and Petersen, 1990
Gobiidae			4.72	Hypoxia-26 d	
<i>Pagothenia borchgrevinki</i>		14.1	2.9	Normoxia	Wells et al., 1989
Notothenidae		27.8	4.7	Hypoxia	
<i>Dormitator latifrons</i> *		41.2	8.1	Wet season-field	Graham, 1985
Eleotridae		33.9	10.5	Hypoxia-14 to 21d	
<i>Fundulus heteroclitus</i>		23.8		Normoxia	Greaney et al., 1980
Cyprinodontidae		36.6		Hypoxia 14 d	
		34.5		Hypoxia 35 d	
<i>Cyprinodon variegatus</i>		~25		Normoxia	Peterson, 1990
Cyprinodontidae		~35		Hypoxia 2.5 h	
<i>Poecilia latipinna</i>	1.2	13.5	2.7	Normoxia	this study
Poeciliidae	1.45	16.3	3.7	Hypoxia 42 d	Peterson, 1990
		~25		Normoxia	
		~28		Hypoxia 2.5 h	

It has been suggested that large increases in hematocrit (and therefore blood viscosity) would incur too great a metabolic cost for cardiac work, which would be particularly expensive for an active species (Wells and Baldwin 1990; Wells and Weber, 1991). Murad et al. (1990) used this argument to explain an increase in the breakdown of pre-existing erythrocytes (karyorrhexis) in *Carrassius auratus* under chronic hypoxia exposure (3 h of acclimation to $0.5\text{--}1.0\text{ mg l}^{-1}\text{ O}_2$). Long-term increases in blood oxygen capacity in *C. auratus* were due to increases in hemoglobin oxygen affinity, facilitated by a decrease in intraerythrocytic adenosine triphosphate (ATP) and possibly through the recruitment of higher affinity isomorphs of hemoglobin (Johansen and Weber, 1976; Weber and Jensen, 1988; Marinsky et al., 1990). In response to chronic hypoxia (72 h of $1.5\text{ mg l}^{-1}\text{ O}_2$), the channel catfish (*Ictalurus punctatus*) did not alter RBC or Hct, but intraerythrocytic adjustments occurred that increased hemoglobin concentration (Scott and Rogers, 1981; Table 3-10). It is possible that an increase in blood viscosity is not as detrimental a factor in *P. latipinna*, a relatively inactive fish with a low routine metabolic rate, as it would be in more active species.

Gallaugh and Farrell (1998) derived a standard curve for the relationship between hemoglobin concentration and Hct for fish species that included a phylogenetically diverse suite of species and covered a range of ecological groupings. The derived relationship accounted for 83% of the variation in [Hb] among fishes. Sailfin mollies acclimated to 6-wk in normoxia and hypoxia exhibited low values of [Hb] and Hct relative to several other fishes, which may reflect their low energetic demands (Figure 3-4, Table 3-10). There are several examples of active fishes that have high Hct and [Hb] values. Active fishes such as skipjack (*Katsuwonus pelamis*) and yellowfin

(*Thunnus albacares*) tunas (Wells et al., 1986) and the striped (*Tetrapturus audax*), blue (*Makaira nigricans*) and black (*Makaira indica*) marlins (Davie, 1990) have much greater Hct and [Hb]. Likewise, several slow-moving and/or benthic fishes seem to have relatively low Hct and/or [Hb] values. These include the sand goby, *Pomatoschistus minutus* (Petersen, 1990, Table 3-7), sheepshead minnow, *Cyprinodon variegatus*, (Haney, 1995), blenny, *Zoarcetes viviparus* (Oikari and Soivino, 1975), and winter (*Pseudopleuronectes americanus*) and starry (*Platichthys stellatus*) flounders (Bridges et al, 1976; Milligan and Wood, 1987).

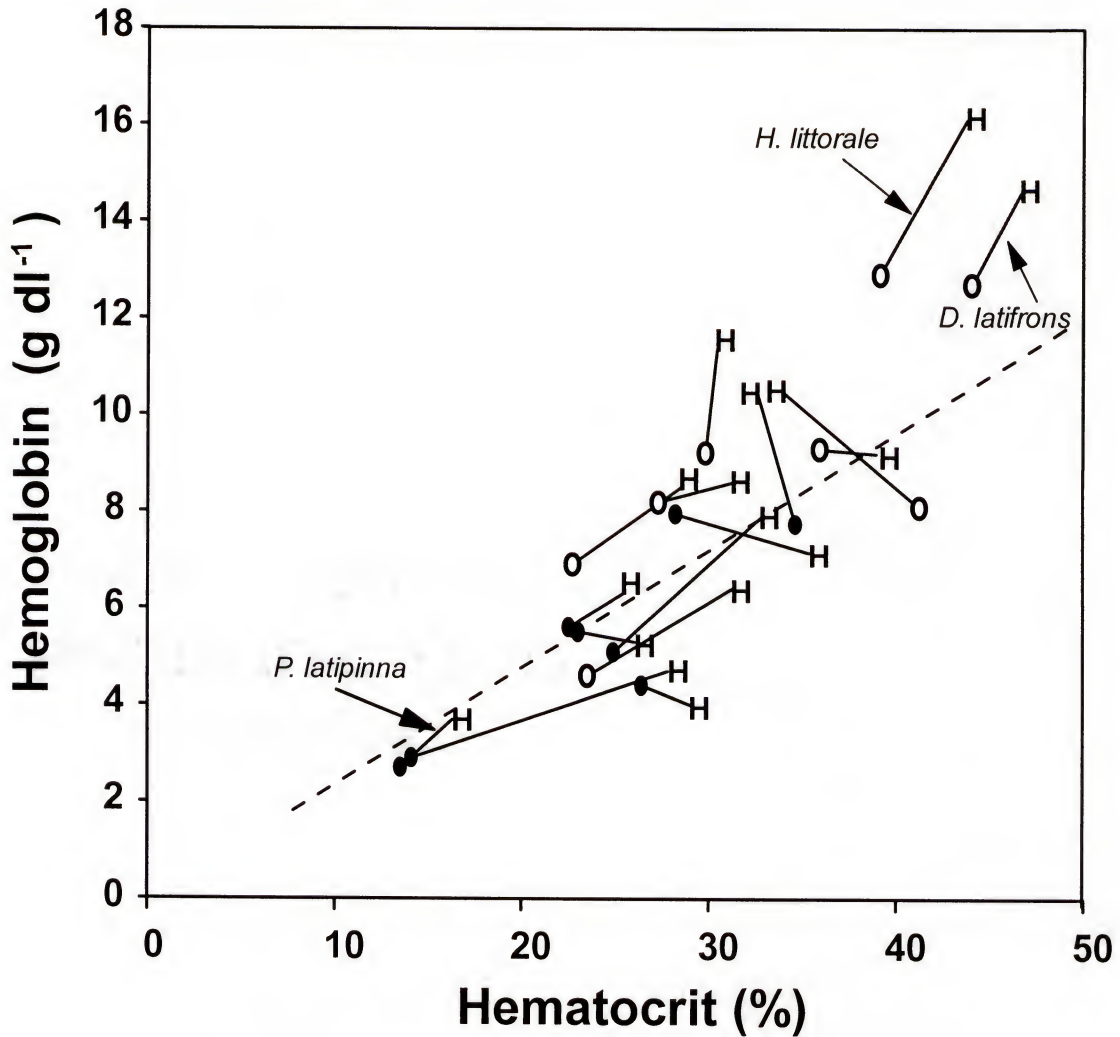


Figure 3-5. The effect of hypoxia acclimation ($H \leq 3 \text{ mg l}^{-1} \text{ O}_2$) on the relationship between hematocrit (%) and hemoglobin concentration (g dl^{-1}) in a diversity of fishes (Weber and Lykkeboe, 1978; Scott and Rogers, 1981; Tetens and Lykkeboe, 1981; Graham 1985; Murad et al., 1990; Wells and Weber, 1990; Val et al., 1990; Val et al., 1992). Open circles indicate facultative air-breathing species. Data plotted here can be found in Table 3-9. The standard curve of the relationship between hematocrit and hemoglobin concentration for fishes based on data presented by Gallagher and Farrell (1998) is included for comparison.

It is interesting to note that some sluggish, facultative air-breathing fishes, such as *Dormitator latifrons* and *Hoplosternum littorale*, have high hemoglobin concentrations and hematocrit (Figure 3-5). Johansen et al. (1978) suggests that their high blood oxygen capacities are probably related to overcoming circulatory inefficiencies associated with their facultative air breathing mode of respiration (Johansen et al., 1978). Temperature also seems to have an effect, albeit small, on intraspecific variation in Hct (Gallaughier and Farrell, 1998). Such complicating factors can obscure the relationship between activity and Hct and [Hb] in teleosts (Wells and Baldwin, 1990). Nevertheless, this suggests that metabolic rate may indeed be one important predictor of the concentration of blood hemoglobin and circulating red blood cells.

Fitness Correlates

Short-term adaptive responses to hypoxia exposure allow fish to cope with environments that are subject to periodic or chronic oxygen scarcity. Although there may be benefits to such environments, such as lower levels of competition for food or shelter or a lower level of predation, exposure to hypoxia may also lead to a reduction in fitness-related characters. In this study, sailfin mollies invoked a suite of both behavioral and physiological responses to cope with the stress of chronic hypoxia that were sufficient to mitigate any detectable negative effect on survivorship. It appears that males are less capable of coping with chronic hypoxia, as they exhibited a 10 to 16% decrease in condition while no significant decrease was found for females. Such gender specific differences in the ability to cope with hypoxia have been previously noted in *P. latipinna* (Peterson, 1990) and other species (e.g., *Gambusia affinis*; Cech et al., 1985). In addition, when female fish were dissected and reproductive state assessed at the end of

each trial, there was no significant difference in the number of actively gestating females between hypoxia and normoxia treatments. Successful gestation occurred throughout hypoxia acclimation and offspring were observed swimming in the aquarium after several weeks of acclimation. The lack of reproductively active females in both hypoxia and normoxia treatments in Trial 4 may reflect a seasonal depression in reproductive activity, although courtship behavior was observed during the acclimation period. Individuals used in this trial were collected late in the breeding season at Cedar Key. A 12L:12D photoperiod, the lower end of the range required for reproduction in *P. latipinna*, (Grier, 1973) was used, and a longer photoperiod may have been required to re-establish reproduction if it had ceased for the year.

Although hypoxia did not affect the number of reproductively active females, or their survivorship, behavioral observations suggest two potential costs of hypoxia exposure. First, the frequency of reproductive behavior in males was lower in hypoxia-acclimated fish during the first three wk of the acclimation period. While this did not result in any detectable difference in the reproductive activity of females, it may impact reproductive success under field conditions, where mollies may be more dispersed. Second, the use of ASR may expose mollies to higher levels of aerial predation. This may be particularly significant for females in late gestation, when their greater metabolic demands may increase time allocated to ASR (Chapter 2). Trexler et al. (1994) showed that an aerial predator of sailfin mollies, the snowy egret, has a preference for large, gravid, females in certain circumstances.

Summary

This study demonstrates that short-term behavioral and physiological responses allow the sailfin molly to cope successfully with extreme hypoxia and suggests an interaction between behavioral response (ASR) and physiological compensation. The use of ASR was an immediate response to hypoxia that decreased over the acclimation period. This supports one hypothesis that gradual physiological compensation (increased [Hb], RBC, Hct) ultimately decreases the threshold for ASR. This was supported further by the decrease in P_c measured in this study. *Poecilia latipinna* exhibited plastic behavioral and physiological responses compensating for hypoxia to a degree that allows them to mitigate diminished condition in females and decreased survivorship in either gender, and that permits continuous reproduction. However, there may be predation costs in the field related to ASR.

Poecilia latipinna is a valuable species for examining the ecological effects of hypoxia on a population. Its widespread distribution in many different types of habitats, from rivers, lakes, and ponds, to swamps and sluggish backwaters of estuaries, provides the natural variation in environmental conditions needed for comparative study (Burgess, 1980). Given that anthropogenically induced hypoxia is an increasing problem, it is important not to ignore the more subtle effects of hypoxia on fishes. Using a species that is more tolerant of chronic hypoxia may be useful in detecting subtle effects of low oxygen stress that may affect species survival.

CHAPTER 4

HYPOXIA AND PHYSIOLOGICAL DIVERSITY: INTERDEMIC VARIATION IN THE RESPIRATORY BIOLOGY OF THE SAILFIN MOLLY (*POECILIA LATIPINNA*)

Introduction

A central issue in physiological ecology is an understanding of how animals respond physiologically to natural variation in the physical environment, how such responses influence the distribution and abundance of populations, and their evolutionary consequences. The physical environment has a major influence on the distribution and movement of organisms. For fishes, the availability of dissolved oxygen is one abiotic factor that exerts a strong selective force in aquatic systems and affects habitat quality, growth, survival, and reproduction (Kramer, 1983a, 1987; Saint-Paul and Soares, 1987; Chapman and Liem, 1995; McKinsey and Chapman, 1998; Secor and Gunderson, 1998; Chapman et al., 1999; Pichavant et al., 2000; Chapter 1). Oxygen scarcity is a common challenge to survival in many aquatic systems characterized by low light and reduced mixing (Chapter 1, Chapter 5; Welcomme, 1979; Chapman and Kramer, 1991; Gessner, 1961; Kramer et al., 1978), Carter and Beadle, 1931; Chapman et. al., 1998; Wetzel, 1975; Truchot and Duhamel-Jouve, 1980; Officer et al., 1984; Justic et al., 1993).

Low oxygen availability has led to the evolution of varied morphological, physiological, and biochemical adaptations in fishes including: the development of air-breathing organs, large gill surface area, increased oxygen carrying capacity of the blood, anaerobic metabolism, low metabolic rate, and morphological specializations for

exploitation of the oxygen-rich surface layer (Lewis, 1970; Galis and Barel, 1980; Liem, 1980; Hochachka, 1993; Kramer, 1983a, 1987; Perry and Laurent, 1993; Perry and McDonald, 1993; Chapman et al., 1995; Graham 1997). Behavioral adaptations permit additional flexibility to deal with temporal and spatial variation in dissolved oxygen and include changes in activity and air-breathing frequency, variation in the use of aquatic surface respiration (ASR), and microhabitat selection (Kramer, 1987; Chapman et al., 1995; Chapman and Liem, 1995). These adaptations have associated costs and benefits that vary with ecological circumstance and affect the ability of fishes to colonize or disperse through oxygen-scarce waters (Kramer, 1983a,b, 1987; Chapman, 1995; Chapman and Liem, 1995). Although much effort has been directed towards describing the physiological and morphological adaptations of fishes to deoxygenation, little is known about the demographic consequences of hypoxic waters.

Strong selection pressure for hypoxia tolerance may lead to interdemic variation among populations with broad habitat ranges. Olowo and Chapman (1996) reported differences in respiratory behavior between populations of the African cyprinid *Barbus neumayeri* from swamp and open water in the same river system. Further study revealed a relationship between total gill filament length and dissolved oxygen concentration (DO) for *B. neumayeri* from six sites in a river drainage in Uganda differing in DO availability (Chapman et al., 1999). Total gill filament length increased as DO decreased, indicating interdemic variation in a morphological trait that correlated with oxygen availability. This interdemic variation may result from differences in the geographical origins of the populations or the developmental and environmental histories of the individuals within the populations.

From the perspective of an ecological physiologist there remain major obstacles that preclude an understanding of the link between physico-chemical stressors and interdemc variation. These obstacles must be addressed in order to determine the source of interdemc physiological diversity. One major issue is the fact that physiological variation occurs at multiple hierarchical levels, i.e., short-term acclimation or acclimatization response, developmental plasticity, genetic variation, and/or their interaction. Historically, experiments have primarily emphasized variation between species or acclimation effects on individuals. Experiments of interdemc variation in eco-physiological characters of fishes are uncommon and have generally ignored the interaction between acclimation effects and fixed or developmental interdemc effects. Previous studies have addressed variation in resistance to chemical toxins (e.g., McCorkle et al., 1979; Diamond et al., 1991; Nacci et al., 1999) or focused on characters like gill morphometry, which are unlikely to be affected by short-term (days to weeks) acclimation response (Chapman et al., 1999, 2000; Chapman and Hulen, in press). These studies did not address interdemc variation in acclimation effects

In this study, I quantified physiological differences in hypoxia compensation between two populations of the sailfin molly, *Poecilia latipinna* (one population from a periodically hypoxic environment and one population from a chronically normoxic environment). Two suites of characters were selected: traits that may show both short-term acclimation response and interdemc variation in acclimation response (metabolic rate, critical oxygen tension, and respiratory behavior), and those that are not likely to respond to short-term acclimation but may vary among populations due to developmental plasticity, genetic effects, and/or their interaction (gill morphometry). To address

hierarchical levels of physiological response, interdemec variation in metabolic rate, critical oxygen tension, and respiratory behavior of sailfin mollies acclimated to normoxia or extreme hypoxia for six wk were quantified. This permits examination of geographical effects on acclimation response.

Methods

Study Site and Species

The sailfin molly (*Poecilia latipinna*) is a widely distributed ovoviviparous species, with populations along coastal areas of the Gulf of Mexico and into the inland fresh waters of Florida (Chapter 1; Burgess, 1980). This euryhaline fish is commonly found in environments characterized by periodically extreme hypoxic conditions, including the shoreline of eutrophic lakes, marshes and wetlands, and estuaries (Trexler and Travis, 1990). It is also found in the well-oxygenated waters of lakes and rivers and in the relatively constant conditions of many Florida springs (Trexler and Travis, 1990; McKinsey and Chapman, 1998).

Sailfin mollies from periodically hypoxic backwaters of the salt marsh of Cedar Key, Florida, were compared to sailfin mollies that inhabit the clear, well-oxygenated, running waters of the Santa Fe River. In the mudflats of the Cedar Key salt marsh, salinity typically fluctuates from near 24 ppt to near 0 ppt. Dissolved oxygen levels are also highly variable (range=0.2-10.8 mg l⁻¹), but extreme hypoxia is not unusual (Chapter 5). At this location, hypoxia was usually associated with low tide and pool isolation, regardless of time of day (Chapter 5). In the Santa Fe River mollies were collected from

the edge of an area of moderate current on three occasions at three sites. In this freshwater habitat, measured oxygen levels were high and showed little variation (mean = 6.75 mg l^{-1} , range = $5.32\text{-}8.20 \text{ mg l}^{-1}$).

Hypoxia Acclimation

Choice of Respiratory Traits

To examine acclimation effects in this study, two integrative measures of hypoxia tolerance and characters were selected that represent synergistic effects of different physiological mechanisms: oxygen consumption and respiratory behavior. Many physiological responses to low dissolved oxygen tension such as increased hemoglobin concentration and lamellar perfusion, can decrease the oxygen tension required to maintain an individual's metabolic rate (critical oxygen tension, P_c), and thus it is not surprising that experiments of acclimation response to hypoxia have detected a decrease in P_c (e.g., Chapter 2). Behavioral response to low oxygen is another integrative measure of hypoxia tolerance (Kramer, 1987). The surface film of water contains high oxygen concentration, and many fish species take advantage of this property by skimming the surface film and passing oxygen-rich water over their gills, a behavior that is referred to as aquatic surface respiration (ASR). Fish with high physiological compensation (increased hemoglobin, gill lamellar perfusion, etc.) tend to have extremely low thresholds (PO_2) for aquatic surface respiration (ASR) and tend to spend less time at the surface once that threshold occurs. Other behavioral traits such as gill ventilation rate, position in the water column, agonism, and reproductive behavior may also change in response to hypoxic stress (Chapter 3).

Experimental Design

Sailfin mollies were collected from the mudflats of Cedar Key and the shoreline of the Santa Fe River and transported to the laboratory in aerated 27-l Nalgene containers. Four 250-l glass aquaria were divided in half with a fine plastic mesh, separating individuals from the two populations (10 fish from Cedar Key (CK) on one side and 10 Santa Fe (SF) mollies on the other side). Care was taken to approximate 1:1 sex ratios in the treatments and aquaria; however, the Santa Fe River collection was skewed towards females, resulting in a final ratio of three males to seven females in each Santa Fe section and five males and five females in the Cedar Key compartments. Once fish were introduced to the aquaria, heaters were used to heat the temperature to 25°C. A salinity of 10 ppt was selected to minimize osmotic stress (Evans, 1975; Nordlie et al., 1992), using a mixture of filtered sea water and distilled water to make up the brackish water of the aquaria. Water temperature and salinity were monitored daily and adjusted as necessary. Two aquaria were exposed to extreme chronic hypoxia ($1 \text{ mg l}^{-1} \text{ O}_2$), and two aquaria were held at normoxia ($7.6 \text{--} 8.2 \text{ mg l}^{-1} \text{ O}_2$) for six wk prior to collection of metabolic rate data. A six-wk acclimation period was chosen based on previous work demonstrating a trade-off between physiological and behavioral response to hypoxia in sailfin mollies from a population subjected to periodic hypoxia. Over a six-wk acclimation period to extreme hypoxia, the use of ASR, an immediate response to hypoxia in sailfin mollies, declined. This suggests more gradual physiological compensation that decreases the threshold for aquatic surface respiration (Chapter 3). A six-wk period facilitated acclimation response in both behavioral and hematological characters and resulted in lowered P_c (Chapter 3). Upon completion of metabolic rate

measurements in each group, fish were re-acclimated to experimental conditions for an additional two wk prior to collection of behavioral data.

A description of the filter systems used in this study is described in Chapter 3. Water-quality measures (e.g., nitrogen, ammonia) were checked frequently over the course of the trials and water changes were made as necessary. The fish were acclimated to a 12L:12D photoperiod and fed Tetra Min flake ® food *ad libitum* twice daily. Fish were allowed to acclimate to these conditions for 7 d prior to beginning experiments. On the 8th day, an aquatic oxygen controller unit (described in Chapter 3) was used to lower progressively the dissolved oxygen level in the hypoxic aquaria to 1.0 mg l⁻¹ O₂ over three-d. Two thirds of the surface area of the hypoxic aquaria was covered by a layer of plastic bubble wrap to minimize oxygen diffusion. This dissolved oxygen level was chosen based on preliminary ASR experiments that showed the 10% ASR threshold for *P. latipinna* occurred near 1.0 mg l⁻¹ O₂. *Poecilia latipinna* experience periodic, fluctuating hypoxia in the Cedar Key salt marsh. This study did not focus on the response to fluctuating dissolved oxygen levels. However, to consider the oxygen consumption rate of field-acclimatized individuals, a second experiment was conducted where females collected from either the Santa Fe River or Cedar Key salt marsh were held under normoxic conditions (as described above) for 3 d prior to metabolic rate and P_c measurements. No males were captured during the field collection at the Santa Fe River for this experiment. Thus, only females were used from both populations. A 3 d period was used to allow fish to recover from transfer from the field to laboratory conditions. These measurements are referred to as estimates of 'field' oxygen consumption rates and P_cs.

Metabolic Rate and Critical Oxygen Tension

The metabolic rate and P_c of each fish was estimated using closed respirometry on post-absorptive fish. Metabolic rate was determined as routine oxygen consumption (RMR; rates during random movement under experimental conditions, Winberg, 1956; Fry 1957) for a range of body sizes. An average of six fish was selected for measurement from each group and trial of the long-term acclimation experiment; fish were selected to maximize the range of body sizes analyzed. For the 'field' estimates, a sample of 10 females was selected from each population. Total RMR was calculated for each fish using data collected at least 30 min after the container was sealed with the probe and prior to estimated P_c .

Each fish was placed in an opaque Nalgene container (130-210 ml) with an air stone and held for 24 h before the trial at 25 °C. At the start of each experiment, the chamber was sealed with a dissolved oxygen probe (YSI Model 600). The meter was programmed to measure water temperature and dissolved oxygen at 10-min intervals and to display plotted values on a portable computer throughout the trial. Once the P_c was detected on the computer-generated plots, the experiment was terminated, and the water in the chamber was aerated and quickly returned to normoxia. Following each trial, the total length and wet weight of the fish were recorded. P_c was determined using the BASIC program by Yeager and Ultsch (1989) that is designed to fit two regression lines to a data set and calculate P_c as the point of intersection of these two lines. RMRs in the six-wk acclimation experiment were measured over a period of 14 d subsequent to the acclimation period with measurements alternated daily between the hypoxia-acclimated

and normoxia-acclimated fish. 'Field' estimates were made within six d of capture, with measurements alternating between individuals from each of the two populations.

For the six-wk acclimation experiment, analysis of covariance (ANCOVA) on log-log transformed data was used to test for effects of body mass on P_c for each treatment within each trial and population. No significant body size effects were detected. T-tests were used to detect trial effects on P_c within treatments and populations. As none were detected, trials were combined for further analyses. Population and treatment effects on P_c were detected using 2-way analysis of variance (ANOVA). Least-squares means were used to test for differences among means when a fixed effect (e.g., treatment) was significant. Previous experiments demonstrated an effect of gestational state on metabolic rate (Chapter 2), but no effect of gender on P_c or RMR (Chapter 2) was noted. Gestating females were removed from analyses, and I did not test for gender effects. ANCOVA revealed no significant trial effects within treatments and populations. Therefore, trials were combined for further analyses. ANCOVA was used to test for effects of population and treatment on total RMR. Bilogarithmic transformation was used to linearize the data and stabilize the variance.

For the 'field' measurements of oxygen consumption, analysis of covariance was used to compare log transformed RMR data from individuals of the Cedar Key salt marsh and the Santa Fe River samples. Any females with late stage embryos (determined by dissection) were removed from analyses. A t-test was used to detect population effects on P_c .

Behavioral Comparisons

Behavioral data were collected for the long-term acclimation experiment only. Once the P_c and RMR data were collected from all groups, fish were allowed to re-acclimate to experimental conditions for another 2 wk. Behavioral data were collected after 10 wk of acclimation. The characters selected included behavioral traits for which I had specific predictions with respect to treatment and population effects based on previous study of the effects of hypoxia on the behavior of *P. latipinna* (Chapter 3). A depression in reproductive behavior, (i.e. courtship displays and copulation attempts) and agonism (i.e. chasing and nipping at fins) under hypoxia and an increase in both ASR and gill ventilation rate was predicted. I also predicted that Santa Fe fish would engage in ASR more frequently, have higher gill ventilation rates, a greater depression in reproductive behavior, and spend more time near the surface in hypoxia than Cedar Key fish.. Rather than collecting behavioral data on each fish individually (as in Chapter 3), a scan approach was used. In this approach, the aquarium was scanned at 5-sec intervals over 10 min period and the number of individuals engaging in a specific behavior were counted. This process was repeated for each behavioral category. The number of individuals conducting ASR was counted, as well as the number of fish involved in agonistic, courtship, or copulatory encounters. The number of courtship and copulatory encounters were counted at once and combined to represent the frequency of reproductive behavior. There was no differentiation between whether the monitored individual was the initiate or the recipient of courtship, copulatory, or agonistic encounters. This scan approach was more appropriate because of interest in population-level differences rather than inter-individual variation. Additionally, because the scan approach integrates

information across many individuals, it reduces the number of zero scores in the data set, increasing the likelihood of observing rare behaviors. In order to estimate how hypoxia affected vertical distribution in the water column, a permanent marker was used to visually divide the water column of each chamber into three equal sections: top, middle and bottom. At the end of each min within a 10-min behavioral data collection session, the level occupied by a majority of the fish was noted. When no majority occupied a single level, dispersion was scored as random. At the end of each 10-min session, individual ventilation rates were assessed by timing the duration required for 20 opercular ventilations for five individuals within each chamber. This entire behavioral data collection process was repeated seven times for each compartment over a period of 10 d. To minimize the effects of dependence among trials, each observation period was separated by at least 24 h, and results were averaged for each 10-min trial.

Subsequent to RMR analyses, a bleach spill resulted in mortality in one normoxia tank. Thus, behavioral data were collected on fish in one normoxia tank (control) and two hypoxia aquaria (treatments). ASR, agonism, and reproductive data exhibited non-normal distributions. Therefore, medians and ranges are reported, and the data were analyzed using non-parametric statistics. The Mann-Whitney U test indicated no trial effects for either population within the hypoxia treatment, so these two trials were combined for further analyses. The Kruskal-Wallis test to detect differences among the four groups (Cedar Key hypoxia, normoxia; Santa Fe hypoxia, normoxia), and Mann Whitney U tests were used for *a posteriori* comparisons. Four sets of *a posteriori* tests were used: Cedar Key normoxia (CKN) versus Cedar Key hypoxia (CKH); Santa Fe normoxia (SFN) versus Santa Fe hypoxia (SFH); CKH versus SFH; and CKN versus

SFN. The Bonferroni correction factor was used to adjust the probability value of significance for multiple comparisons to 0.013, and 1-tailed tests were used to calculate p values. Spatial distribution data were plotted as the mean percent frequency of occurrence.

Gill Morphometry

Fish from each population were preserved in paraformaldehyde (35 g l⁻¹). Total gill filament length was measured using standard methods modified after Muir and Hughes (1969) and Hughes (1984). For each fish, the left branchial basket was removed, and the four gill arches were separated. For each hemibranch of the gill arches, the length of every fifth gill filament was measured. Two successive measurements along a hemibranch were averaged and multiplied by the number of filaments in the section between the two filaments. Filament lengths were summed for the four hemibranchs and multiplied by two to produce an estimate of total gill filament length (TFL). Lamellar density was measured in the dorsal, middle, and ventral parts of every tenth filament of the second gill arch on the left side. The total number of lamellae (on one side of the filament) and average lamellar density (ALD) were estimated using a weighted mean method that takes into account the difference in length of various filaments (Muir and Hughes, 1969; Hughes and Morgan, 1973). For every tenth or twentieth filament (depending on the number of filaments), the lengths of the secondary lamellae were measured a number of times over the filament (Galis and Barel, 1980). Lamellar height was also measured on specimens where it could be accurately determined. Average values of these characters for each filament were converted to estimates of lamellar area using a regression determined through the dissection of 30 to 60 lamellae from various

sections of the second gill arch from two or more specimens from each population. The sum of the total lamellar area for all sections of the second arch was divided by the total number of lamellae and multiplied by two to produce a weighted average bilateral surface area on one side of the filament (ALA). Total gill surface area (TGA) was determined using the formula: $TGA = TFL \times 2 \times ALD \times ALA$.

Analysis of covariance (ANCOVA) was used to compare total gill filament length and total gill surface area between the Santa Fe and Cedar Key populations of *P. latipinna* with body mass (total weight of preserved specimens) as the covariate. All variables were \log_{10} transformed. Adjusted means (sample means adjusted for a common mean body mass and a common regression line) were calculated from the ANCOVA analysis. It has been recommended that the reduced major axis method be applied to structural relationships such as those presented in this chapter (Ricker, 1984; McArdle, 1987). However, the ordinary least squares regression was used because there was no reason to believe that the error rate of the X variable (body mass) was more than a third that of the Y variable (gill character) (McArdle, 1987). In addition, many of the relationships between the gill characters and body size had correlation coefficients greater than 0.9, which means that there is little difference expected between the regression lines produced by the two methods (Ricker, 1984; McArdle, 1987).

Results

Behavior

Poecilia latipinna collected from Cedar Key were consistently more aggressive than their Santa Fe conspecifics (CKN versus SFN, $p=0.005$, CKH versus SFH, $p<0.001$, Figure 4-1). However, acclimation to chronic hypoxia had no effect on the level of aggression in either population (CKN versus CKH, $p=0.052$; SFN versus SFH, $p=0.072$, Figure 4-1). Reproductive behaviors were less frequent in the Santa Fe groups than Cedar Key groups (CKN versus SFN, $p=0.003$; CKH versus SFH, $p<0.001$, Figure 4-1). In addition, acclimation to chronic hypoxia reduced the frequency of reproductive behaviors in both populations (CKN versus CKH, $p=0.005$; SFN versus SFH, $p=0.008$, Figure 4-1). Aquatic surface respiration (ASR) did not occur in normoxia for individuals from either population. However, the median frequency of ASR was higher for hypoxia-acclimated fish from the Santa Fe River than for those from Cedar Key (SFH versus CKH, $p=0.003$, Figure 4-1). Santa Fe mollies regardless of acclimation, appeared to spend more time in the top one third of the aquaria than Cedar Key mollies. This percentage increased for Santa Fe mollies under hypoxic conditions (SFN= 7.14 ± 3.95 SE; SFH= 20 ± 3.08 SE; CKN= 2.86 ± 1.84 SE; CKH= 2.5 ± 1.37 SE; Figure 4-3). Gill ventilations were not quantified in normoxia-acclimated fish because opercular movement in normoxia was too subtle to permit an accurate count. Under hypoxic conditions, Santa Fe River individuals had a much higher gill ventilation rate than Cedar Key fish (Figure 4-2, $p<0.001$).

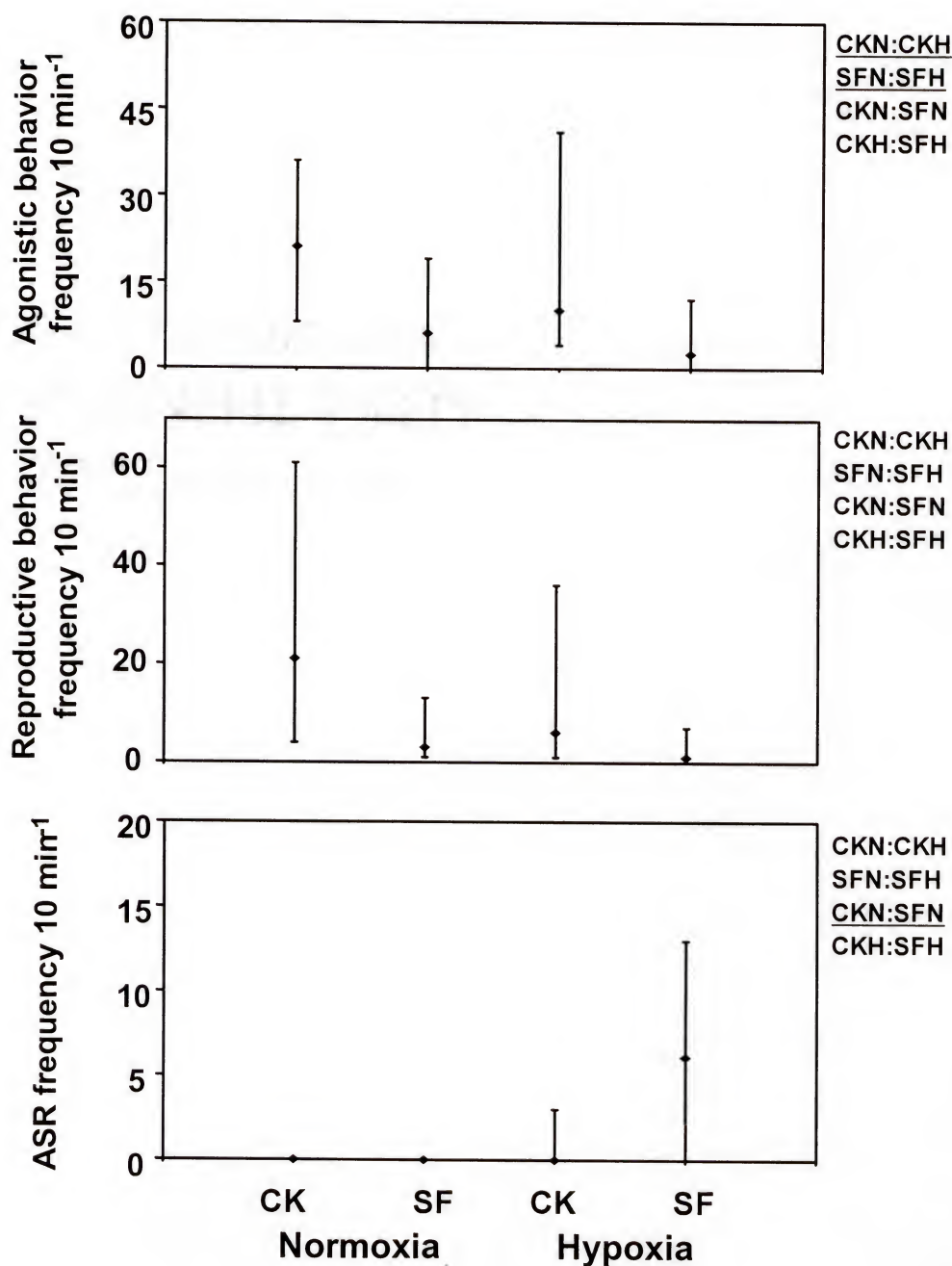


Figure 4-1. Median (diamonds) and range (vertical lines) for the frequency of agonistic behavior, reproductive behavior, and aquatic surface respiration for sailfin mollies (*Poecilia latipinna*) from the Cedar Key salt marsh (CK: periodically hypoxia) and the well-oxygenated waters of the Santa Fe River (SF). Mollies were acclimated for at least 10 wk in normoxia ($7.8\text{--}8.3\text{ mg l}^{-1}\text{ O}_2$) or 10 wk in extreme hypoxia ($1.0 \pm 0.10\text{ mg l}^{-1}\text{ O}_2$). Paired comparisons underlined were not significantly different. ($P < 0.05$).

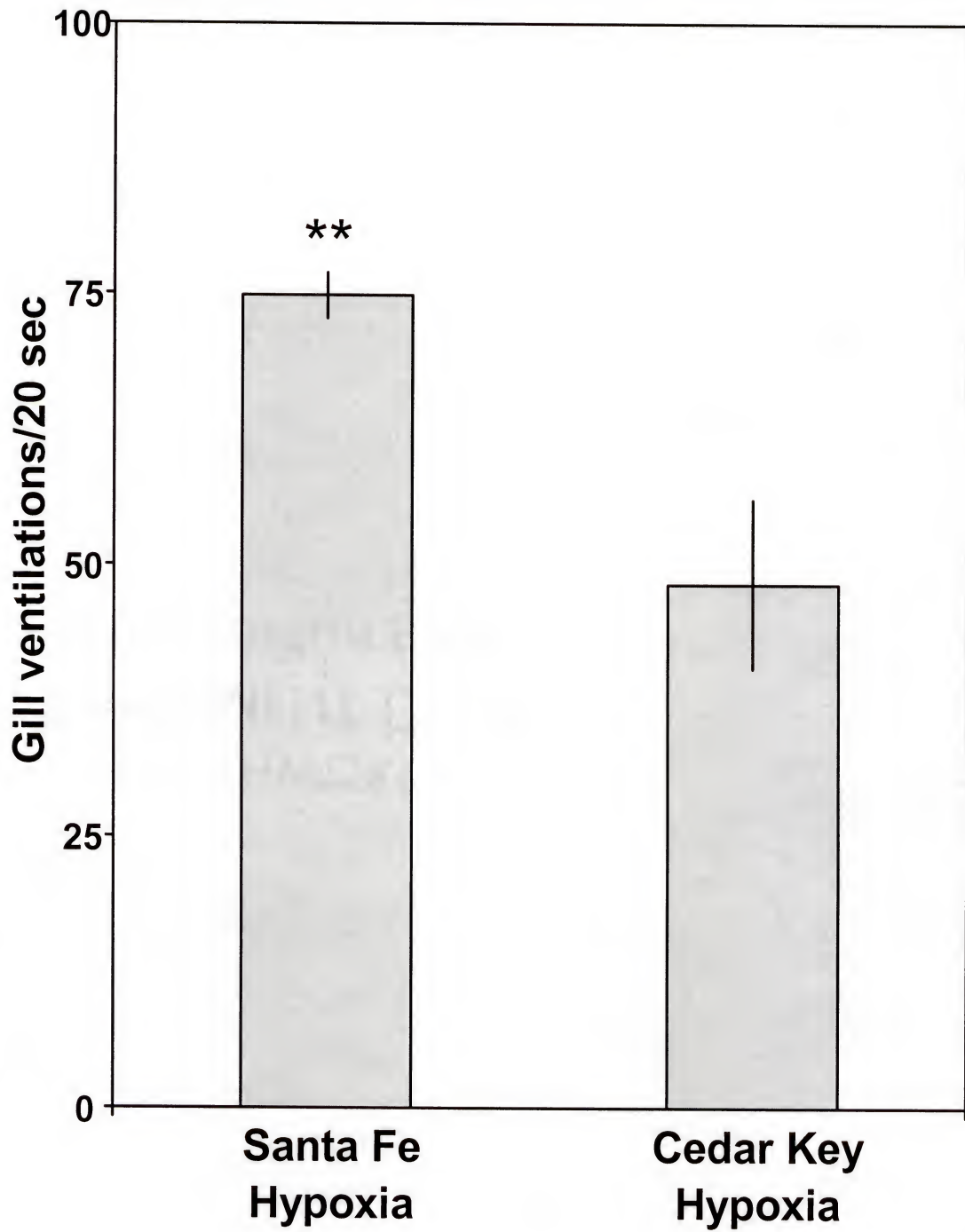


Figure 4-2. Number of gill ventilations per 20-sec interval for sailfin mollies (*Poecilia latipinna*) from the Cedar Key salt marsh (periodically hypoxia) and the well-oxygenated waters of the Santa Fe River. Mollies were acclimated for at least 10 wk in extreme hypoxia ($1.0 \pm 0.10 \text{ mg l}^{-1} \text{ O}_2$). ** = $p < 0.001$.

Mollies from Cedar Key spent less time at the bottom and more time in the upper third of the water column under hypoxia acclimation (Figure 4-3). Fish from the Santa Fe River population showed the same trend but exhibited a far more dramatic increase in the time spent in the upper third of the water column during hypoxia. This may reflect the higher proportion of time allocated to ASR in the Santa Fe River fish than in the Cedar Key population.

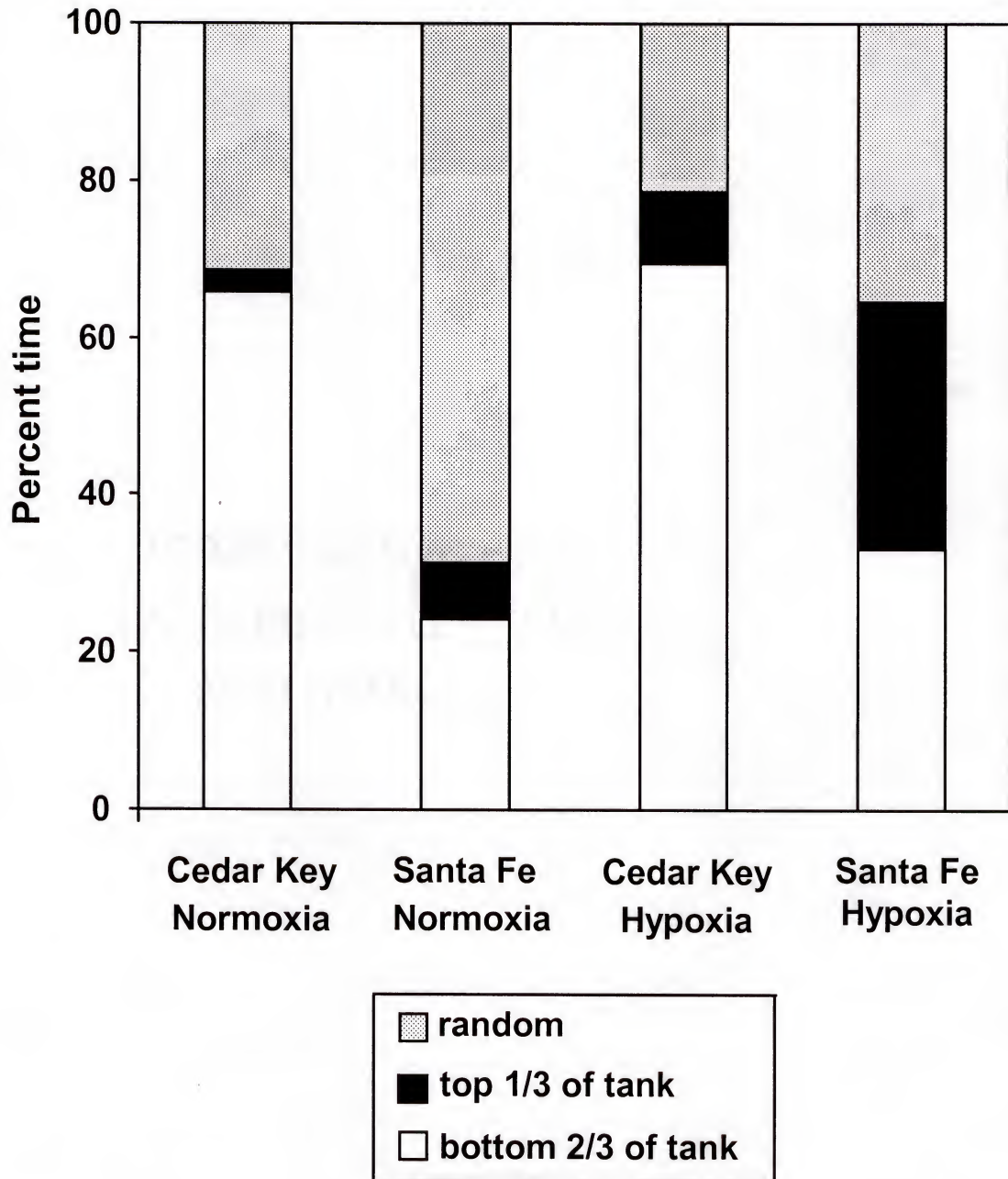


Figure 4-3. Vertical distribution of sailfin mollies (*Poecilia latipinna*) in experimental aquaria expressed as a frequency of occurrence (%). Fish were derived from two populations, the periodically hypoxic Cedar Key salt marsh and the well-oxygenated Santa Fe River, and acclimated to normoxia ($7.8\text{--}8.3\text{ mg l}^{-1}\text{ O}_2$) or extreme hypoxia ($1.0 \pm 0.10\text{ mg l}^{-1}\text{ O}_2$) for more than 10 wk.

Metabolic rate and Critical Oxygen Tension

There was no significant effect of either population or acclimation treatment on the bilogarithmic relationship between RMR and body mass (Table 4-1; Table 4-2). In addition, 'field' oxygen consumption estimates did not differ significantly between populations (slope: $F=0.000$; $p=0.995$; intercept: $F=0.243$, $p=0.628$).

However, P_c differed among populations and treatments (Table 4-3). Cedar Key mollies appeared better able to cope with extreme hypoxia and have P_c values consistently lower than Santa Fe River mollies regardless of acclimation (Table 4-3, Figure 4-4). 'Field' P_c values exhibited the same pattern, with fish from Cedar Key exhibiting significantly lower P_c values than those collected from the Santa Fe River ($t=-2.20$, $p=0.021$). Both populations exhibited lower P_c values after acclimation to chronic hypoxia (Table 4-3, Figure 4-4). However, the effect was greater for the Cedar Key population, where P_c was 30% lower after acclimation to hypoxia; in the Santa Fe River population, P_c exhibited a 19% decline.

Table 4-1. Mean weight, standard error, body mass range, estimated marginal mean routine metabolic rate (RMR) and range of sailfin mollies (*Poecilia latipinna*) from the Cedar Key salt marsh (CK) and the Santa Fe River (SF) acclimated to normoxia ($7.8\text{--}8.3\text{ mg l}^{-1}\text{ O}_2$) or extreme hypoxia ($1.0 \pm 0.10\text{ mg l}^{-1}\text{ O}_2$). 'Field' measurements were taken after three-d of acclimation to normoxic laboratory conditions.

Trial	Treatment	Pop.	N	Body mass mean \pm SE (g)	Body mass range (g)	mean RMR \pm SE mg O ₂ hr ⁻¹	RMR range mg O ₂ hr ⁻¹
1	Normoxia	CK	9	2.69 ± 0.342	0.92-4.07	0.197 ± 0.025	0.09-0.60
		SF	4	$0.82 \pm 3.86\text{E-}02$	0.72-0.91	0.190 ± 0.029	0.08-0.19
	Hypoxia	CK	6	2.99 ± 0.692	0.54-4.58	0.261 ± 0.038	0.13-0.86
		SF	7	$0.94 \pm 5.82\text{E-}02$	0.83-1.23	0.220 ± 0.022	0.09-0.20
2	Normoxia	CK	3	3.37 ± 0.78	2.29-4.88	0.280 ± 0.059	0.36-0.81
		SF	6	0.74 ± 0.13	0.31-1.12	0.257 ± 0.043	0.10-0.25
	Hypoxia	CK	5	3.50 ± 0.36	2.29-4.36	0.318 ± 0.058	0.45-1.07
		SF	5	$0.73 \pm 6.63\text{E-}02$	0.56-0.88	0.278 ± 0.049	0.08-0.23
3	Field	CK	10	3.26 ± 0.376	1.63-6.02	0.53 ± 0.039	0.32-0.83
		SF	10	2.58 ± 0.424	1.19-4.63	0.54 ± 0.039	0.22-0.83

Table 4-2. ANCOVA showing the effects of acclimation treatment (normoxia versus hypoxia; DO), population (P), and their interaction on the relationship between \log_{10} metabolic rate and \log_{10} weight in sailfin mollies (*Poecilia latipinna*). Fish were collected from two populations, the periodically hypoxic Cedar Key salt marsh and the well-oxygenated Santa Fe River, and acclimated to normoxia ($7.8\text{--}8.3\text{ mg l}^{-1}\text{ O}_2$) or extreme hypoxia ($1.0 \pm 0.10\text{ mg l}^{-1}\text{ O}_2$) for at least six wk.

Source of Variation	Df	MS	F	p-value
Log weight	1	0.943	52.087	<0.001
Population (P)	1	0.000	0.000	0.991
Dissolved oxygen (DO)	1	0.054	2.988	0.092
P x DO	1	0.008	0.458	0.503
Error	40	0.086		

** mean body mass= 1.46 g

Table 4-3. ANOVA showing population and acclimation effects (hypoxia versus normoxia; DO) and their interaction on the critical oxygen tension of sailfin mollies (*Poecilia latipinna*). Fish were derived from two populations, the periodically hypoxic Cedar Key salt marsh and the well-oxygenated Santa Fe River, and acclimated to normoxia ($7.8\text{--}8.3\text{ mg l}^{-1}\text{ O}_2$) or extreme hypoxia ($1.0 \pm 0.10\text{ mg l}^{-1}\text{ O}_2$) for at least six wk.

Source of variation	Df	MS	F	p-value
Population (P)	1	1.645	13.306	0.001
Dissolved oxygen (DO)	1	0.765	6.187	0.018
P x DO	1	0.078	0.631	0.433
Error	35	0.178		

**mean body mass = 1.52 g.

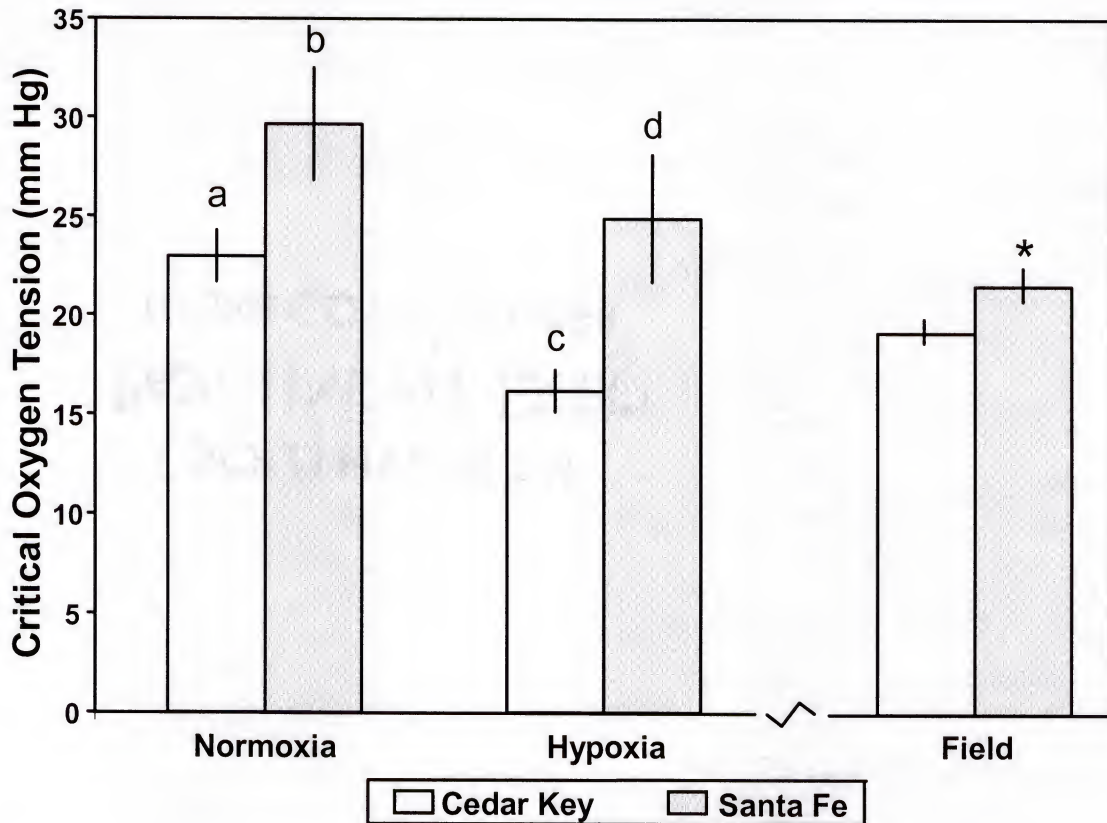


Figure 4-4. Critical oxygen tension (mm Hg) for sailfin mollies (*Poecilia latipinna*) from the periodically hypoxia waters of the Cedar Key salt marsh and from the well-oxygenated waters of the Santa Fe River. Fish from both populations were acclimated to either to normoxia ($7.8\text{--}8.3\text{ mg l}^{-1}\text{ O}_2$) or extreme hypoxia ($1.0 \pm 0.10\text{ mg l}^{-1}\text{ O}_2$) for six wk prior to measurements. Bars showing different letters are significantly different (2-way ANOVA, $p < 0.05$). Critical Oxygen tension measurements taken from individuals recently collected from these sites (field) are also included for visual comparison and $*=p < 0.05$ for field groups.

Gill Morphometry

Gill characters were measured on eight fish from each population selected to maximize range in body size (Cedar Key: mean body mass=2.08 g, range=0.92 to 5.00 g; Santa Fe: mean=2.09 g, range=1.06 to 4.87 g). Gill measurements permitted estimation of the following characters: total gill filament length, average lamellar density, average lamellar area, and total gill surface area.

For the two populations, total gill filament length, average lamellar area, and total gill surface area were positively correlated with body size (Table 4-4). Average lamellar density was negatively related to body size in both groups (Table 4-4). ANCOVA indicated no difference in the slopes of the bilogarithmic relationships between individuals of the Cedar Key and Santa Fe River populations for any of the gill characters examined (Table 4-4). However, intercepts differed for two of the four gill morphometric characters (Table 4-4). When adjusted for body mass, total gill filament length was greater in fish from the periodically-hypoxic waters of the Cedar Key salt marsh than in fish from the well-oxygenated waters of the Santa Fe River (Table 4-4, Figure 4-5). The longer gill filament length among individuals in the Cedar Key population was reflected in larger gill surface area. Total gill surface area was 14% greater in fish from the Cedar Key salt marsh population than in those from the Santa Fe River population (Table 4-4, Figure 4-6). Neither lamellar density nor average lamellar area differed between the two populations.

Table 4-4. Summary of linear regression analyses and analyses of covariance of relationships between gill morphometric characters and body mass (g) for sailfin mollies (*Poecilia latipinna*) from the Cedar Key salt marsh (periodic hypoxia) and the well-oxygenated waters of the Santa Fe River. Both gill characters and body mass were \log_{10} transformed. The mean values represent antilogged adjusted means calculated from the ANCOVA analyses (sample means adjusted for a common mean body mass of 2.08 g and a common regression line).

Character	Site	Slope	Intercept	R	p value	ANCOVA F	Slope p value	ANCOVA F	Intercept p value	Adjusted means
Total gill filament length (cm)	CK	0.488	2.037	0.967	<0.001	0.094	0.765	10.186	0.007	155.96
	SF	0.518	1.978	0.972	<0.001					138.36
Lamellar density (no. per mm)	CK	-0.084	1.693	0.930	0.001	1.358	0.267	2.717	0.123	46.34
	SF	-0.059	1.677	0.818	0.013					45.50
Lamellar area (mm ²)	CK	0.196	-2.496	0.850	0.007	4.101	0.066	0.387	0.545	0.0037
	SF	0.080	-2.469	0.788	0.020					0.0036
Total gill surface area (cm ²)	CK	0.611	0.831	0.924	0.001	0.436	0.522	5.653	0.033	10.62
	SF	0.529	0.791	0.959	0.000					9.12

Palzenberger and Pohla (1992) reviewed literature on gill morphology in freshwater fishes. Based on 28 species of non-air breathing freshwater fishes, they calculated the mean slope of the bilogarithmic plot of total gill surface area and body mass. They set the lowest and highest mean values within each parameter range to 0 percent and 100 percent, respectively, to create a range of values for each parameter. Using their mean slope and the range of intercepts for the fishes in their study, data on *P. latipinna* were converted into a percentage of the estimated range of all species summarized by Palzenberger and Pohla (1992) for total gill surface area. Fish from the periodically hypoxia waters of the Cedar Key salt marsh had values for gill surface area averaging 44% of the range of freshwater fishes. In contrast, gill surface area values for fish from the well-oxygenated waters of the Santa Fe River averaged only 36% of the range of freshwater fishes.

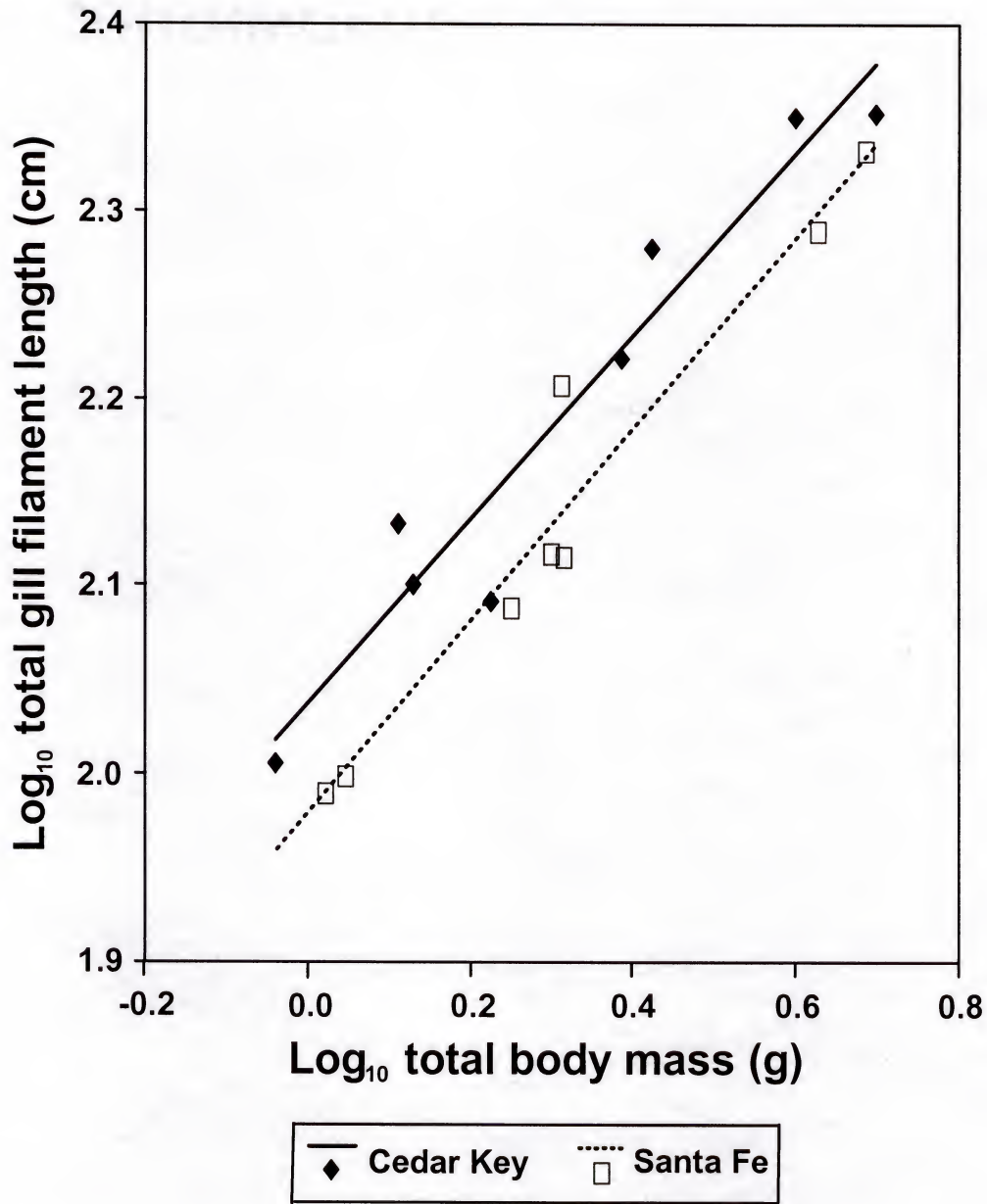


Figure 4-5. Bilogarithmic plot of the relationship between total gill filament length (cm) and body mass for sailfin mollies (*Poecilia latipinna*) from the periodically hypoxic waters of the Cedar Key salt marsh, and the well-oxygenated waters of the Santa Fe River. ANCOVA indicated a significant difference in the adjusted mean total gill filament length between the two populations ($p=0.007$).

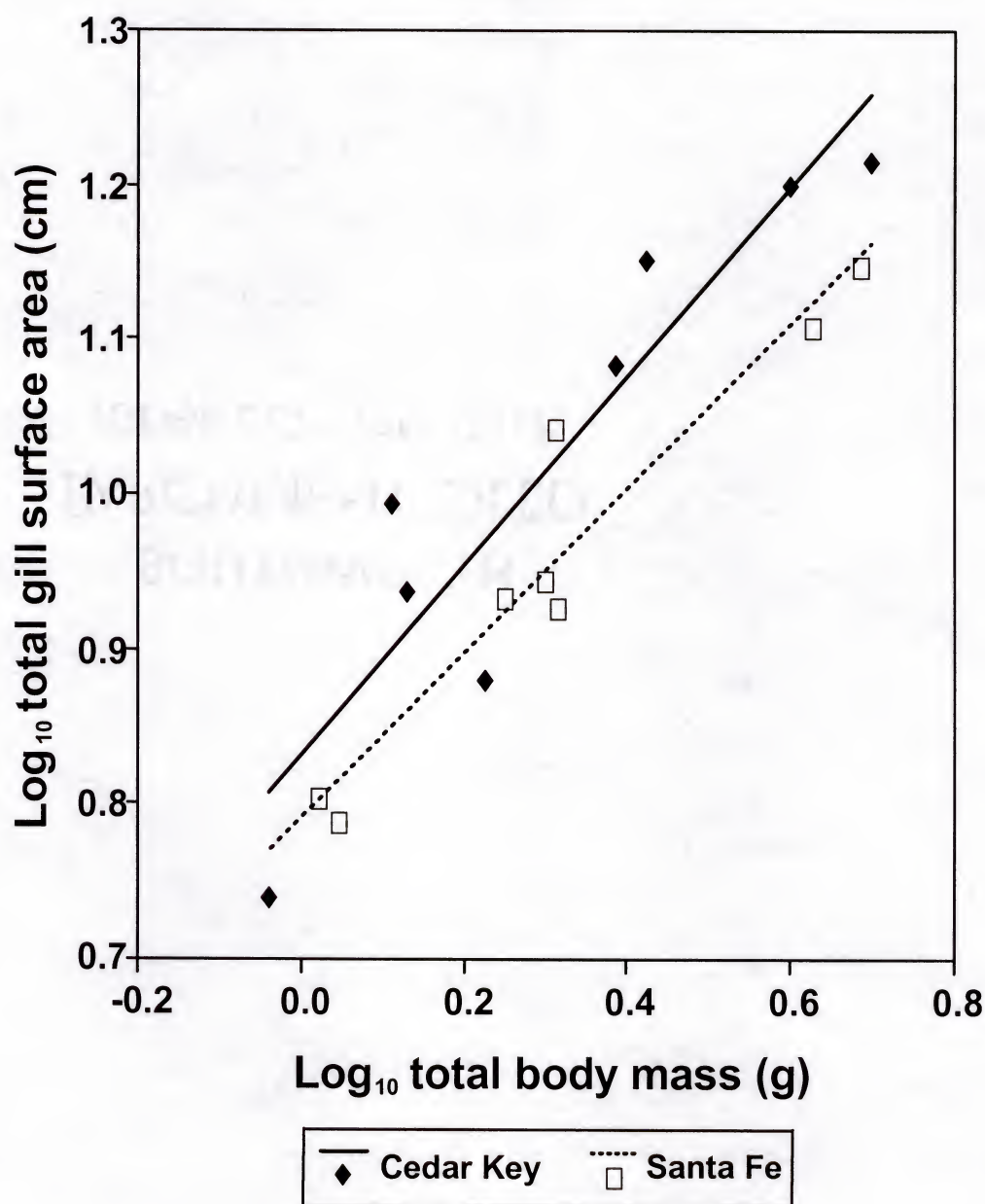


Figure 4-6. Bilogarithmic plot of the relationship between total gill surface area (cm^2) and body mass for sailfin mollies (*Poecilia latipinna*) from the periodically hypoxic waters of the Cedar Key salt marsh, and the well-oxygenated waters of the Santa Fe River. ANCOVA indicated a significant difference in the adjusted means of total gill surface area between the two populations ($p=0.033$).

Discussion

In fishes, plastic behavioral and physiological responses to hypoxia may be critical for species living in habitats characterized by strong spatial and temporal variation in dissolved oxygen content. In addition, strong selection pressure for hypoxia tolerance in oxygen-scarce habitats may lead to interdemic variation among populations with broad habitat ranges. This interdemic variation may derive from differences in the geographical origins of the populations or the developmental and environmental histories of the individuals within the populations. The results of this study of *P. latipinna* demonstrate short-term acclimation response to hypoxia, interdemic variation in acclimation response, and interdemic variation in gill morphometry. The source of the observed interdemic variation is unknown but supports a strong role of hypoxic waters in physiological diversification.

Short-term Acclimation Response

Acclimation to chronic hypoxia indicated short-term compensatory response, but also demonstrated interdemic variation in the acclimation response. Individuals from both populations spent more time near the surface and more time conducting ASR under hypoxia. However, sailfin mollies from the Cedar Key salt marsh, a population frequently exposed to severe hypoxic events, spent less time conducting ASR, had lower gill ventilation rates, and spent less time near the surface than conspecifics from the Santa Fe River, a well-oxygenated site. In fact, time allocated to ASR was extremely low in the

Cedar Key population, even under extreme hypoxia. This may reflect selection pressure to minimize time spent at the surface under hypoxia and the development of other mechanisms to cope with hypoxic stress.

Poeciliids appear generally well-suited to survive hypoxic conditions, having a dorsally flattened head and an obliquely upturned mouth that allows them to efficiently skim the oxygen rich-water surface layer while maintaining a horizontal swimming position (McKinsey and Chapman, 1998). Short-term experiments have shown that, without access to the surface, mollies sustain high mortality rates at low oxygen levels (Peterson, 1990), suggesting that ASR is a primarily mechanism for coping with hypoxia. ASR is clearly a widespread behavioral response to extreme aquatic hypoxia in fishes (Gee et al., 1978; Kramer and McClure, 1982; Saint-Paul and Soares, 1987; Gee and Gee, 1991; Chapman et al., 1994, 1995; Val and de Almeida-Val, 1995; Olowo and Chapman, 1996; Chapman and Chapman, 1998). However, it has been shown to have high associated energetic costs and can increase risk of aerial predation (Kramer et al., 1983). Therefore, fish adapted to periodic or chronic hypoxia may have developed other physiological, morphological, or biochemical mechanisms to reduce the need for ASR or to lower their ASR threshold. In Chapter 3, a decrease in the ASR frequency in *P. latipinna* from the Cedar Key salt marsh occurred with acclimation time, suggesting that there are costs to the behavioral response that favor physiological compensation. Lower allocation to ASR in the Cedar Key population, when compared to the Santa Fe River population, may reflect the greater development of other mechanisms to cope with hypoxia, such as their larger gill surface area, or perhaps hematological adjustments (Chapter 3). The lower gill ventilation rates in the Cedar Key fish also support a higher

tolerance to hypoxia and may reflect interdemic variation in characters such as gill size. Olowo and Chapman (1996) found variation in gill ventilation rate between swamp and open-water populations of a small cyprinid, *Barbus neumayeri*. Swamp-dwelling fish, characterized by larger gills (Chapman and Liem, 1995, Chapman et al., 1999) exhibited lower gill ventilation rates under extreme hypoxia than open-water fish with small gills.

Acclimation to hypoxia also reduced the frequency of reproductive behaviors and increased the time spent conducting ASR in individuals of both populations. However, Cedar Key individuals exhibited higher rates of reproductive behaviors than Santa Fe River individuals. This may reflect interdemic variation in reproductive behavior, a phenomenon that has been shown to occur among other populations of this species (Farr and Travis, 1989; Ptacek and Travis, 1998). However, since there were fewer males in the Santa Fe River groups, differences in sex ratio between the experimental groups cannot be ruled out as a factor influencing reproductive behavior rate.

Poecilia latipinna from Cedar Key acclimated to normoxic conditions exhibited a lower P_c than similarly acclimated fish from the Santa Fe River. Fish from both the Cedar Key and Santa Fe River populations had lower P_c values after acclimation to chronic hypoxia, but Cedar Key individuals consistently exhibited lower P_c values than those from the Santa Fe River, regardless of acclimation, thus demonstrating interdemic variation in acclimation response. The mean P_c of Santa Fe River individuals immediately following collection from the field was lower than for those acclimated to normoxia for six wk, perhaps indicating that fish from the Santa Fe River may occasionally be exposed to low oxygen conditions. The mean P_c values of fish immediately following collection from the Cedar Key salt marsh was intermediate

between the mean P_c values of mollies acclimated to six wk of chronic hypoxia or normoxia. This may be expected because the fish were exposed to chronic hypoxia in this experiment, while mollies experience only periodic hypoxia in the field at Cedar Key.

Several experiments have examined interdemc variation in tolerance of fishes (measured as survivorship) to physical factors such as salinity (e.g., *P. latipinna*, Nordlie et al., 1992; *Gadus morhua*, Nelson et al., 1996); pH (*Perca flavescens*, Nelson and Mitchell, 1992), and resistance to pesticides (*Gambusia affinis*, McCorkle et al., 1979; *Fundulus heteroclitus*, Nacci et al., 1999). Studies of interdemc variation in physiological characters are rare but have demonstrated differences in oxygen consumption between cave-dwelling and surface-dwelling populations of *Astyanax fasciatus* (Characidae; Hueppop, 1986) and land-locked, brackish-water, and open-ocean populations of Atlantic cod (*Gadus morhua*; Nelson et al., 1994, 1996). Interdemc variation has also been noted in exercise physiology of the Atlantic cod (Nelson et al., 1994). Experiments examining variation among populations in hypoxia tolerance or physiological characters affecting hypoxia tolerance in fishes are extremely rare, but have provided evidence for interdemc effects. For example, Bel'chenko and Kel (1992) found that *Carrassius auratus* collected from a lake experiencing regular hypoxia had a significantly higher blood volume per kg body mass, as well as a higher hemoglobin concentration per kg body mass, than individuals from a normoxic site. Interdemc effects on blood oxygen capacity have been shown in yellow perch (*Perca flavescens*) populations in lakes of differing pH (Nelson et al., 1988). When McCorkle et al. (1979) compared the hypoxia tolerance of a pesticide-resistant population of *Gambusia affinis* to a pesticide-susceptible population, they found that the pesticide-resistant population to

exhibit reduced tolerance. It is possible that *G. affinis* with characters leading to superior hypoxia tolerance, such as increased gill surface area, might have experienced higher pesticide susceptibility due to increased uptake through the gills, thus selecting against more hypoxia-tolerant individuals.

In this study of *Poecilia latipinna*, the source of the interdemec variation in acclimation response characters was not identified. Interdemec variation could be genetically based, fixed by environmental pressures at a critical period of ontogeny, or simply be a phenotypic response that remains labile throughout an individual's lifetime. A study of clonal strains of *Daphnia pulex* from a eutrophic pond showed genotypic effects on characters affecting hypoxia tolerance, including metabolic rate and hemoglobin concentration (Weider and Lampert, 1985). In a long-term acclimation study, Nordlie et al. (1992) increased salinity over several weeks to determine salinity tolerance differences in freshwater and estuarine populations of sailfin mollies (*P. latipinna*). After weeks of progressively increasing salinity, the estuarine population was able to tolerate 80 ppt while the freshwater population tolerated 70 ppt. Nordlie et al. (1992) showed that salinity tolerance is a plastic character, but that population-level differences were still maintained. Similar experiments on *P. latipinna* have shown that populations living in either mildly brackish or freshwater habitats (with a threshold of 4 ppt) are distinct in their response to temperature and salinity. Travis and Trexler (1987) suggested that these differences have a genetic basis, but emphasized the need for quantitative data. In the current study of *P. latipinna*, both populations were able to reduce their P_c in response to hypoxia, but population level differences were still maintained after six wk of acclimation. This suggests that, although P_c is a plastic

character, there are other developmentally plastic or genetic differences between the two populations. A rigorously controlled growth experiment would be useful in teasing apart the relative environmental and genetic contributions to interdemographic variation in P_c .

Interdemographic Variation in Gill Morphometry

The physiological and behavioral interdemographic variation observed in the acclimation response of sailfin mollies suggests that there are other developmentally plastic or genetic differences between populations in characters compensating for hypoxia tolerance. The 14% larger gill surface area in fish from the Cedar Key population is one character that may contribute to the observed pattern, as a larger gill surface area should increase oxygen uptake capacity.

Studies of interdemographic variation in morphological characters of fishes have focused on jaw, skull, and body morphology, and several have demonstrated significant morphological differences between or among populations (Wimberger, 1991, 1992; Toline and Baker, 1993; Day et al., 1994; Motta et al., 1995; Holopainen et al., 1997; Mittelbach et al., 1999; Cutwa and Turingan, 2000; Sediva et al., 2000). Studies of interdemographic variation in gill morphometry are few but also support variation in gill size among populations of some species. For example, Chapman et al. (1999) quantified the relationship between total gill filament length and dissolved oxygen concentration (DO) for the African cyprinid *Barbus neumayeri* among populations from sites that differed in dissolved oxygen availability and found a negative relationship between total gill filament length and dissolved oxygen concentration. Chapman and Hulen (in press) recorded a greater total gill surface area in a swamp-dwelling population of the African mormyrid *Gnathonemus victoriae* than for fish from an open-water populations, and similar results

were documented in the African cichlid, *Pseudocrenilabrus multicolor victoriae* (Chapman et al., 2000). The degree to which interdemic variation in gill morphometry represents phenotypic plastic and/or genetic differences in these species and for *P. latipinna* is unknown. However, Chapman et al. (2000) raised *P. multicolor* from a single brood under normoxic and hypoxic conditions to detect plasticity in gill size. Total gill surface area was 18% greater in the hypoxia- acclimated group, demonstrating plasticity in gill size, but the difference between field populations was greater (38%) and suggesting both genotypic and phenotypic effects. A study of the decapod *Munida quadrispina* showed that gill size is developmentally plastic and responds to exposure to chronic hypoxia. Populations of *M. quadrispina* subjected to chronic severe hypoxia nearly doubled their ratio of gill dry weight to dry body mass compared to counterparts in normoxic waters (Burd, 1988).

Trexler and Travis (1987) determined that *P. latipinna* exhibits a trend toward increased genetic differentiation with distance between populations, conforming to a model of isolation by distance. At a distance of 51 km, genetic differences between two populations are likely to be greater than genetic variability within each population. The distance between Cedar Key and the site where mollies were collected on the Santa Fe River ($\cong 78$ km) may be sufficient to prevent significant genetic mixing and allow for the development of habitat-associated genetic distinctions, although many other factors, not measured, may be involved.

It seems likely that the increased gill surface area seen in individuals from the Cedar Key population is related to dissolved oxygen availability. However, the two sites in question differ not only in oxygen availability but also in salinity. The Cedar Key

population is exposed to salinity values ranging from near zero (on rare occasions) to 24 ppt (daily), while the Santa Fe River population is in a spring-fed (hardwater) river.

Differences in osmoregulatory requirements between the two populations might also be a factor in the observed gill size differences. An increase in the gill surface area devoted to chloride cells may impede respiratory uptake of oxygen (Bindon et al. 1994).

Circumstantial evidence indicates that these cells function in salt transfer processes, in both fresh and saltwater. Under osmotically stressful conditions, the surface area of the gill devoted to chloride cells may increase (Evans, 1993). In one study, the apical surface area of individual branchial filament chloride cells, number of these cells, and their apical surface density per unit of filament epithelial surface area were examined in *O. mykiss*, exposed to Strasbourg tapwater, seawater, or ion-poor water. Chloride cell proliferation only increased significantly in the ion-poor water (Binden et al. 1994). It is possible sailfin mollies could react similarly, with essentially no difference in the number or size of chloride cells between hard freshwater populations and estuarine populations.

Alternatively, the osmotically variable environment of the estuary might be sufficiently stressful to induce an increase in gill surface area allocated to chloride cells, resulting in a compensatory increase in gill size for estuarine mollies. This is apparently supported by Palzenberger and Pohla (1992) who summarized that the gill areas of freshwater fish are smaller than those of comparable marine species, a trend that could not be adequately explained by activity level alone. A controlled growth experiment using salinity and hypoxia as treatments would be helpful in teasing apart the relative contribution of each of these factors to variation in gill morphometric characters.

Summary

Despite great interest in the evolution of physiological and morphological adaptations to deoxygenation, the impact of hypoxic waters on diversification is not well understood. The results of this study suggest that hypoxia may potentially act as a strong selective force contributing to interdemec variation in physiological, behavioral, and morphological characters in *P. latipinna*. Sailfin mollies from the Cedar Key salt marsh, a population frequently exposed to severe hypoxic events, spent less time conducting ASR, had lower gill ventilation rates, and spent less time near the surface than conspecifics from the Santa Fe River, a well-oxygenated site. *Poecilia latipinna* from Cedar Key acclimated to normoxic conditions exhibited a lower P_c than similarly acclimated fish from the Santa Fe River. Acclimation to chronic hypoxia caused both populations to decrease their P_c values, but differences between the two populations were maintained. Cedar Key fish had a 14% higher mean gill surface area relative to fish from the Santa Fe River, a character that could account, at least in part, for their greater tolerance to hypoxia. The source of these interdemec differences remains to be determined and will be important to understanding of the role of hypoxic waters in the maintenance and promotion of physiological diversity.

CHAPTER 5

PATTERNS OF HYPOXIA IN A COASTAL SALT MARSH: IMPLICATIONS FOR THE ECOPHYSIOLOGY OF RESIDENT FISHES

Introduction

Effective uptake of dissolved oxygen is critical to the long-term survival of most aquatic organisms. However, there are many systems where water may not remain saturated with oxygen, leading to hypoxia. Hypoxia generally occurs in systems characterized by low light and reduced mixing, such as heavily vegetated swamps, (Carter, 1934; Carter, 1955; Carter and Beadle, 1931; Chapman et al., 1998, in press; Chapman and Liem, 1995); flooded forests (Gessner, 1961; Kramer et al., 1978, Chapman and Chapman, in press); floodplain lakes and ponds (Welcomme 1979; Junk et al., 1983); and the deep waters of lakes and ponds (Wetzel, 1975; Lewis and Weibezahn, 1976; Rai and Hill, 1981; Kizito et al., 1993). Anoxia or severe hypoxia can also occur when seasonally or chronically deoxygenated waters in deep-water habitats periodically well up into shallow habitats (e.g., Lake Victoria: Hecky, 1993; Chesapeake Bay: Breitburg, 1990).

Variation in mixing (due to wind, tide or gravitational circulation), density stratification, and quality of water flowing into an area can affect variation in the duration, frequency, and extent of hypoxia (Diaz et al., 1992). Strong seasonal variation in dissolved oxygen occurs in many systems associated with seasonal fluctuations in rainfall, mixing, incident light, and water temperature. In intermittent tropical streams,

habitats may shift from fast flowing, well-oxygenated habitats in the wet season to small isolated hypoxic pools in the dry season (Chapman and Kramer, 1991a). Spatial variation in oxygen is also evident as strong vertical gradients in many deep lakes (Rai and Hill, 1981; Kizito et al., 1993) and in horizontal gradients in lakes with marginal areas covered by floating vegetation (Bonetto et al., 1969; Junk et al., 1983; Saint-Paul and Soares, 1987). Diurnal patterns of reduced oxygen availability, linked to photosynthetic and respiratory activity have been observed in many shallow water habitats, such as eutrophic lakes, tropical pools (Chapman and Kramer, 1991b), and many estuarine habitats (Officer et al., 1984; Rosenberg et al., 1992; Justic et al., 1993; Rabalais et al., 1998). These waters can range from near anoxia at dawn, caused by bacterial and plant respiratory uptake of oxygen, to oxygen supersaturation at the height of photosynthetic activity in the afternoon (Kramer et al., 1978).

The pattern of hypoxia in coastal marshes is particularly complex due to changes in tidal flow, seasonal variation, and complex spatial variation in patterns of mixing. In addition, tidal influences and seasonal changes in rainfall result in strong fluctuations in other physico-chemical characters, most notably, salinity (Odum, 1988). Although shallow waters of tidal marsh systems are typically well mixed and have high photosynthetic productivity, hypoxia can occur (Subrahmanyam and Drake, 1975). The receding tide may lead to hypoxia in isolated pools during the day, where metabolic oxygen uptake may exceed photosynthetic production. In addition, solar input may heat these pools to over 40°C (Subrahmanyam and Drake, 1975), and evaporation may increase salinity (>35 ppt, Kilby, 1955), thus significantly reducing the waters ability to hold oxygen. Hypoxia is also possible during tidal isolation at night, when

phytoplankton and plants consume oxygen. In addition, there is growing evidence that many coastal marine ecosystems are severely impacted by the increase of nutrients loadings from land. Eutrophication of these coastal waters accelerates the factors leading to hypoxia and anoxia, resulting in more frequent and longer hypoxic episodes (Rosenberg et al., 1992).

Although, there has been much interest in the tolerance of coastal salt marsh fishes to extreme physico-chemical conditions (*Cyprinodon variegatus*, Nordlie, 1985; Haney, 1995; *P. latipinna*, Nordlie et al., 1992), there are few studies that document patterns of hypoxia and other water-quality parameters in these systems. In this study, I examined temporal and spatial patterns of hypoxia in the Cedar Key salt marsh of northwestern Florida and identifying key predictors of dissolved oxygen availability. I also report spatial and temporal variation in water temperature and salinity. Finally, I discuss the implications of these patterns for the ecology and physiology of resident fishes.

Study Site

The tidal marsh of Cedar Key lies where the flat shoreline of the upper peninsula of Florida meets the shallow waters of the Gulf of Mexico (Figure 5-1). Distinguished from terrestrial habitats by tidal submergence, the tidal marsh hosts a diverse and characteristic biota. The shoreline waters near Cedar Key consist of coastal salt marsh that are more saline than fresh and extend about 5 km in width. The dominant emergent macrophyte, *Spartina alterniflora* fringes many of the oyster bars and outlying small islands. Dense thickets of black mangrove, *Avicennia nitida*, as well as oyster grass, *Spartina alterniflora* dominate the landscape. Closer to the mainland, black rush, *Juncus*

roemerianus, also occurs. A sinuous maze of channels links the shallow back-waters of these mudflats to the deeper coastal waters of the Gulf. At extremes in tide, the entire tidal area may be completely submerged or consist of expanses of bare, flocculent ooze, interspersed with islands of vegetation and isolated pools. Those animals living in the inner salt marsh must survive extremes in temperature and salinity.

Aquatic oxygen conditions may become stressful in the mudflats of Cedar Key, when many inland pools become isolated, creeks become shallow or dry with receding tides, and aquatic temperatures exceed 35°C (Kilby, 1955). Under these conditions, even non-benthic species may face hypoxic stress.

Methods

Measurements of dissolved oxygen, water temperature, and salinity were taken at four sites in the Cedar Key salt marsh. Sites were selected to include both pool and channel sites and represent areas where *Poecilia latipinna* were collected for Chapters 2, 3, and 4 (Figure 5-1). These sites were located at a large culvert 1.2 km from the town of Cedar Key on Florida State Highway 24. Site 1, a pool, was more isolated than the other sites, especially during low tide. Sites 2, 3 and 4 appear to have more runoff from terrestrial freshwater sources and were isolated from each other only on occasional extreme low tide events. Since it was predicted that tide might be an important factor in determining hypoxia, measurements were taken twice every three months (one with a morning low tide, one with a morning high tide) to produce six quarters of data. To describe diel variation, measurements at four sites were taken every two hours from 08:00 to 17:00. Salinity was measured using an optical refractometer. Dissolved oxygen

concentration and water temperature were measured using a YSI Model 57 meter. The average of two dissolved oxygen readings taken 10 cm from the surface of the water was used to estimate aquatic oxygen levels.

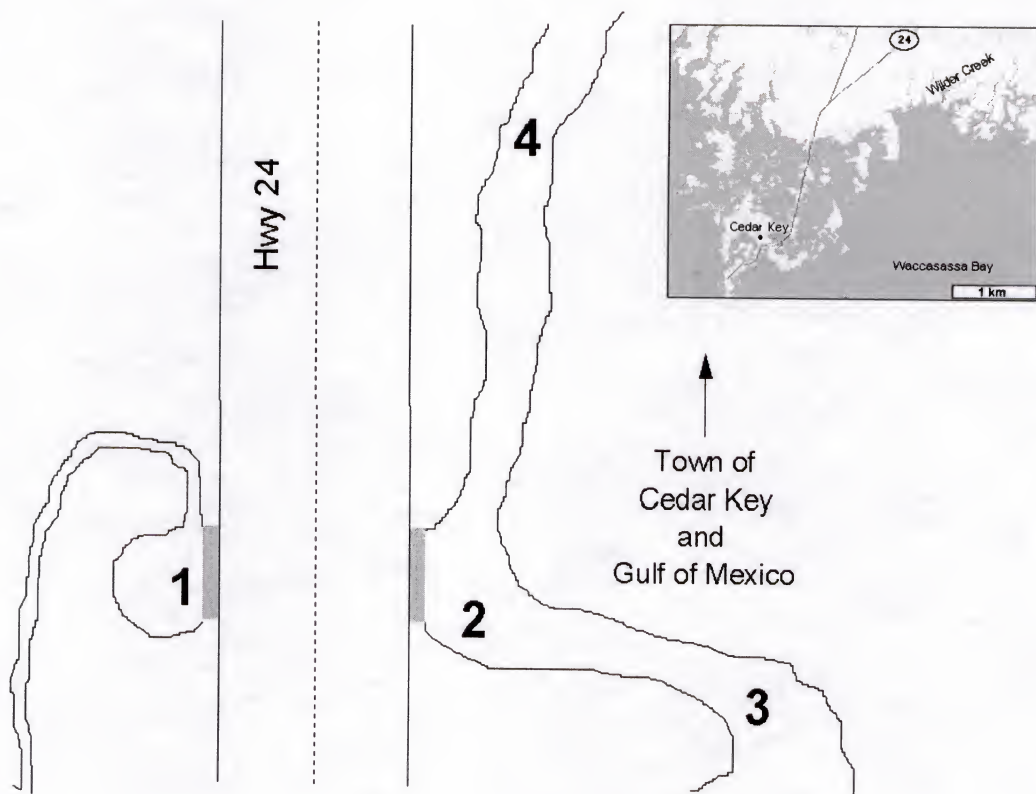


Figure 5-1. Map of sites where dissolved oxygen concentration, salinity, and water temperature were measured at the salt marsh of Cedar Key.

To test for inter-site variation in mean oxygen levels, the mean dissolved oxygen concentration over each sample day was calculated for each site producing 13 values across 21 months of sampling. Paired t-tests were used to detect differences in the mean dissolved oxygen levels between pairs of sites. The Bonferonii correction factor was used to adjust the P-value of acceptance for the multiple comparisons to $p=0.008$. To test for a significant seasonal trend in mean dissolved oxygen levels, a repeated measures analysis was conducted on the first year of data with the four sites representing the replicates. Data collected in morning low tide and morning high tide were analyzed separately. Helmert contrasts were used to identify when a sample period differed from the previous sample.

Results

There were consistent differences between the physico-chemical characters measured at Site 1 and those of other sites (Figure 5-1) despite their relative proximity. Mean dissolved oxygen level at Site 1 was lower than at the other three sites (paired t-tests: Sites 1 vs 2: $t=-4.362$, $p=0.0014$; 1 vs 3: $t=-4.276$, $p=0.0016$, 1 vs 4: $t=-4.409$, $p=0.0012$, Figure 5-2, Table 5-1). The oxygen levels at sites 2 through 4 were highly correlated (2 vs. 3: $r=0.995$, $p<0.000$; 2 vs 4: $r=0.996$, $p<0.000$; 3 vs 4: $r=0.993$, $p<0.000$), and no differences in mean levels were detected between any two pairs of the three sites (paired t-tests, $P>0.05$, Figure 5-2).

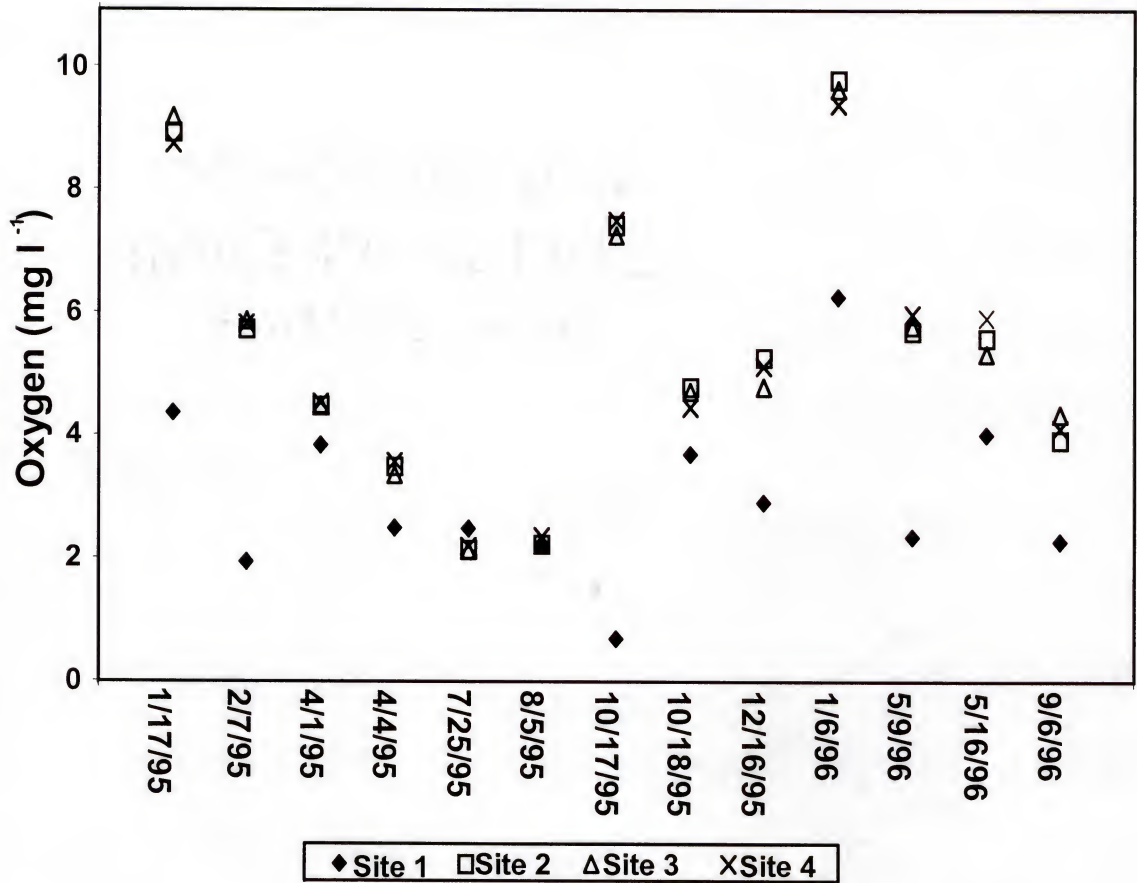


Figure 5-2. Average oxygen concentration (mg l⁻¹) measured over a diel cycle for each of four sites measured in the Cedar Key salt marsh.

Dissolved oxygen varied significantly among sampling dates (Figure 5-2). During the first 12 months of sampling, mean dissolved oxygen values collected when the morning tide was low were highest in the summer (mean=7.1 mg l⁻¹) and lowest in the spring (mean=2.9 mg l⁻¹, repeated measures ANOVA, F=50.681, p<0.001, repeated contrasts, p<0.05). For samples collected when the low tide was in the afternoon, the lowest mean dissolved oxygen level was also recorded in the spring (mean=2.23 mg l⁻¹), but the highest mean was recorded in late fall (mean=8.4 mg l⁻¹, repeated measures ANOVA, F=32.86, p=0.008, repeated contrasts, p<0.05).

Dissolved oxygen showed very strong diurnal variation on many of the sample days ranging by an average of 3.8 mg l⁻¹ for Site 1, 3.5 mg l⁻¹ for Site 2, 3.5 mg l⁻¹ for Site 3, and 3.3 mg l⁻¹ for Site 4 (Figures 5-3 and 5-4). Although extreme hypoxia (<1.0 mg l⁻¹ oxygen) occurred at all sites, it was more common at Site 1 than at the other sites. Minimum levels averaged 1.5 (range = 0.2 to 3.5) for Site 1; 3.4 (range = 0.5 to 9) for Site 2; 3.5 (range = 0.4 to 9) for Site 3; and 3.6 (range = 0.4 to 8.5) for Site 4. These seasonal and spatial trends observed for dissolved oxygen concentration were very similar when percent oxygen saturation was used. In many cases, an afternoon low tide appeared to depress the mean oxygen level for the day (Figures 5-3 and 5-4). The lowest oxygen levels recorded in a diurnal cycle were often associated with morning low tides and dissolved oxygen levels appeared to follow the ebb and flow of the day's tidal currents (e.g., Figure 5-5). In a few cases, however, the diurnal pattern of dissolved oxygen availability appeared opposite of tide (e.g., Figure 5-6).

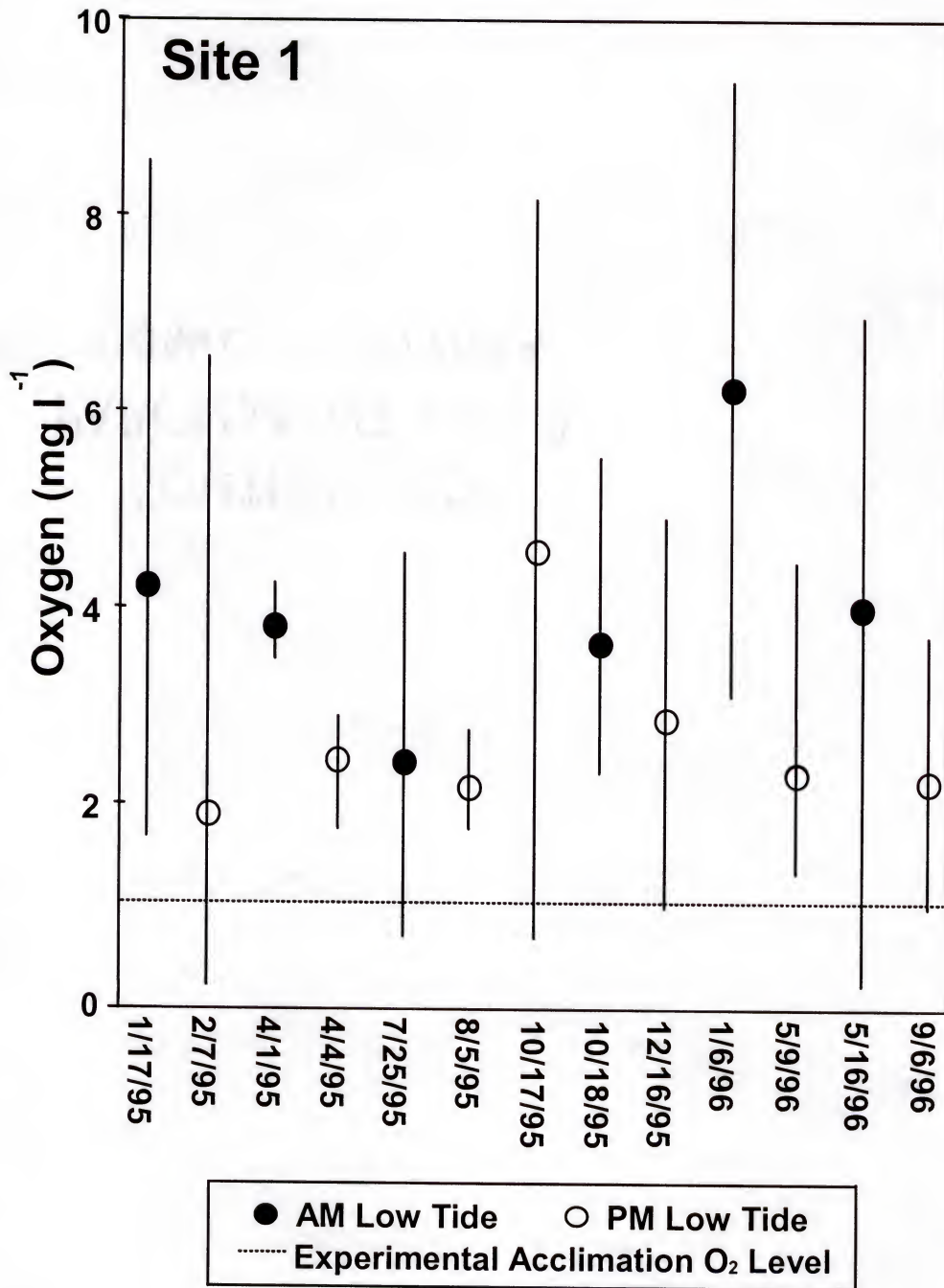


Figure 5-3. The daily mean and range of dissolved oxygen concentration (mg l^{-1}) at Site 1 in the Cedar Key salt marsh. The 1 mg l^{-1} dissolved oxygen level is indicated as the acclimation level selected for hypoxia experiments in Chapters 3 and 4.

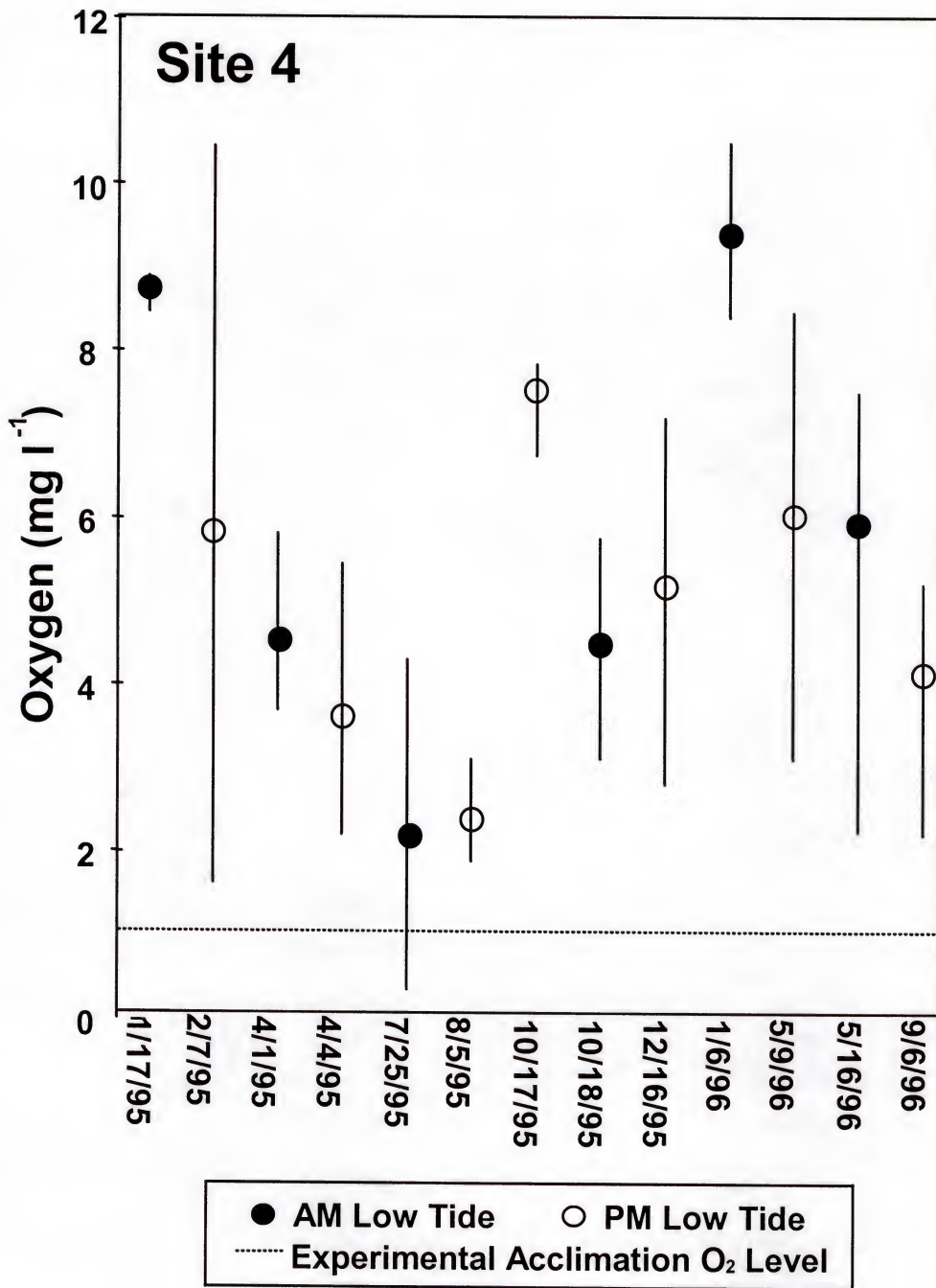


Figure 5-4. The daily mean and range of dissolved oxygen concentration (mg l^{-1}) at Site 4 in the Cedar Key salt marsh. The 1mg l^{-1} dissolved oxygen level is indicated as the hypoxia acclimation level selected for experiments in Chapters 3 and 4.

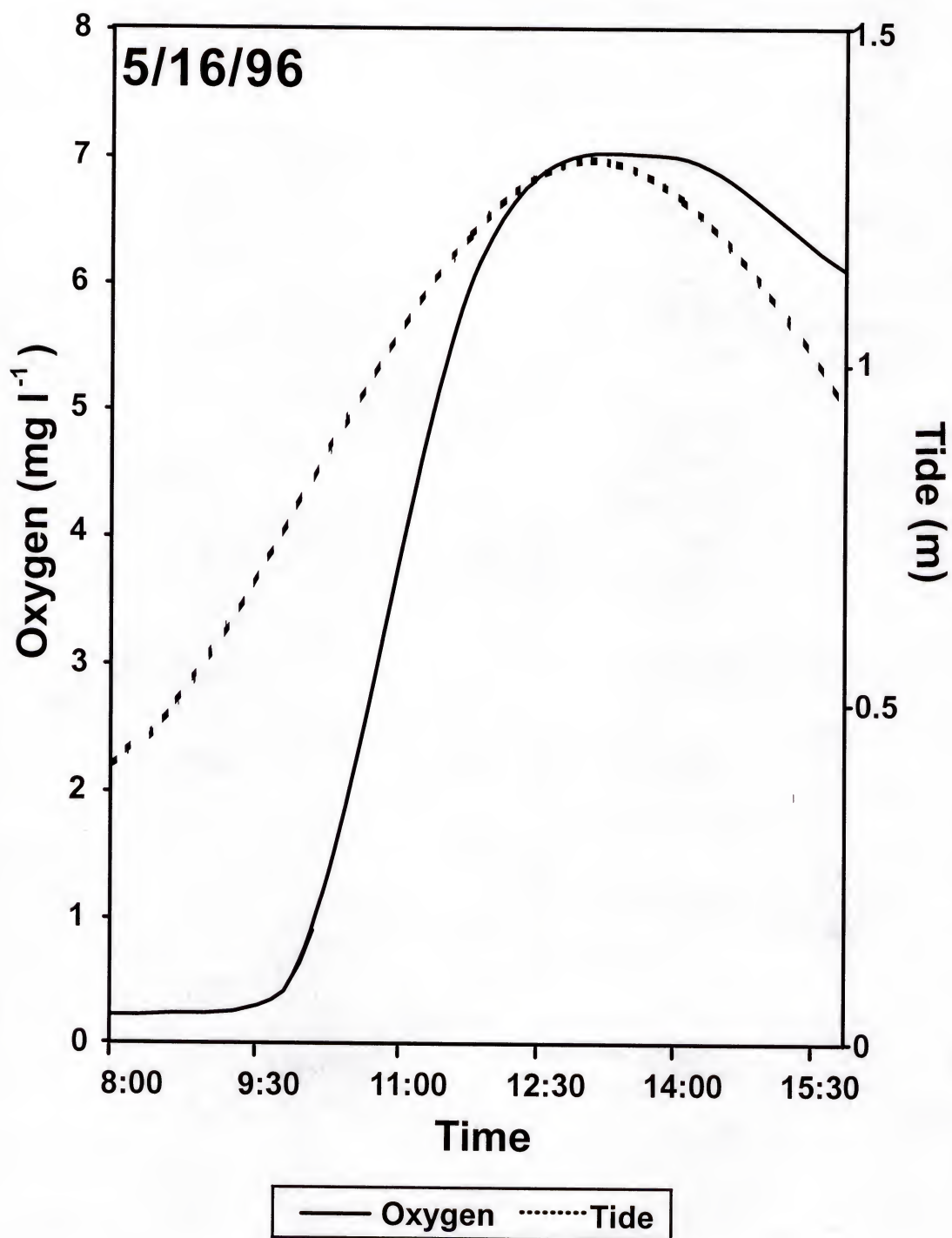


Figure 5-5. The patterns of dissolved oxygen concentration (mg l^{-1}) and tide height (m) for 5/16/96 at Site 1 in the Cedar Key salt marsh.

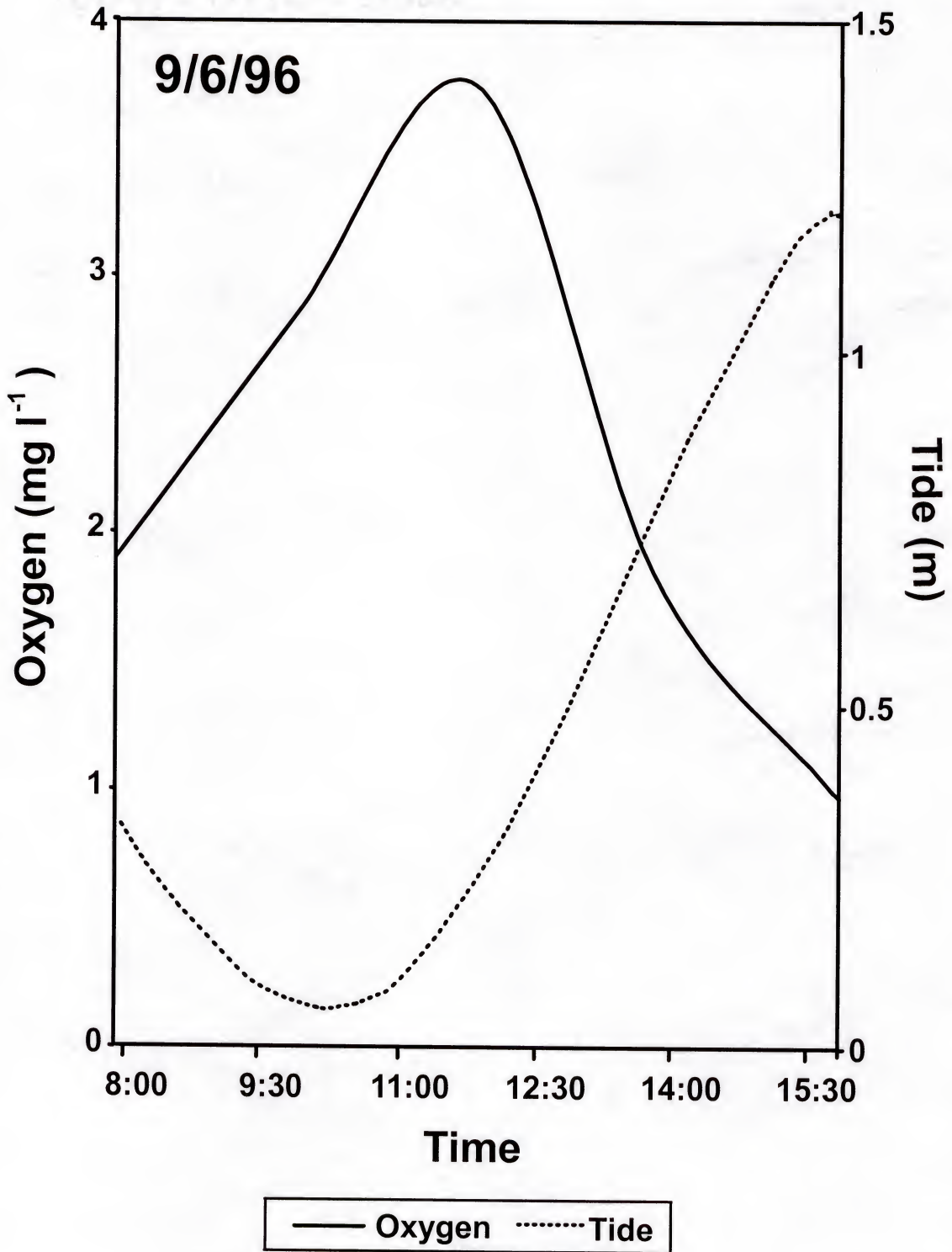


Figure 5-6. The patterns of dissolved oxygen concentration (mg l^{-1}) and tide height (m) for 9/6/96 at Site 1 in the Cedar Key salt marsh.

Table 5-1. Dissolved oxygen concentration (mg l^{-1} , daily mean and range) measured at four sites of Cedar Key salt marsh.

Date	Morning Tide	Site 1 Oxygen (mg l^{-1})	Site 2 Oxygen (mg l^{-1})	Site 3 Oxygen (mg l^{-1})	Site 4 Oxygen (mg l^{-1})
1/17/95	Low	4.36 1.9-8.5	8.92 8.7-9.0	9.17 8.8-9.6	8.73 8.4-8.9
2/7/95	High	1.93 0.2-6.6	5.73 1.1-10.6	5.86 1.2-10.3	5.81 1.6-10.4
4/1/95	Low	3.83 3.53-4.3	4.47 3.4-5.8	4.49 3.6-5.6	4.53 3.7-5.8
4/4/95	High	2.49 1.8-2.9	3.49 2.1-5.6	3.35 2.0-5.1	3.56 2.2-5.4
7/25/95	Low	2.48 0.7-4.6	2.14 0.5-4.1	2.12 0.4-4.1	2.19 0.3-4.3
8/5/95	High	2.20 1.8-2.8	2.22 1.6-3.1	2.22 1.6-3.1	2.35 1.8-3.1
10/17/95	High	4.6 0.7-8.2	7.42 6.4-8.1	7.25 6.3-8.1	7.49 6.7-7.8
10/18/95	Low	3.69 2.3-5.6	4.79 3.6-5.8	4.71 3.4-5.9	4.45 3.1-5.7
12/16/95	High	2.90 1.0-4.9	5.27 2.8-7.4	4.79 2.6-6.3	5.12 2.8-7.2
1/6/96	Low	6.25 3.1-9.4	9.78 9.0-10.8	9.62 8.9-10.6	9.38 8.4-10.5
5/9/96	High	2.34 1.3-4.4	5.69 3.0-8.3	5.78 2.9-8.4	5.97 3.1-8.4
5/16/96	Low	4.01 0.2-7.0	5.59 1.8-7.4	5.33 1.2-7.5	5.92 2.2-7.5
9/6/96	High	2.27 1.0-3.7	3.92 2.1-5.8	4.34 2.1-5.9	4.11 2.2-5.2

Water Temperature and Salinity

Water temperature varied little among sites (Site 1: mean daily average=23.48 °C, mean daily range=19.8-27.13°C; Site 2: mean daily average=23.2°C, mean daily range=19.6-26.9°C; Site 3: mean daily average=23.4°C, mean daily range=19.8-28.5°C; Site 4: mean daily average=23.0°C, mean daily range=18.9-26°C, Table 5-2).

Differences in salinity among sites were greater than temperature differences and seemed to be affected by differential inland freshwater (FW) run-off among the sites (Site 1: mean daily average=15.1 ppt, mean range=11.2-19.9 ppt; Site 4: mean daily average=13.5 ppt, mean range=9.0-18.8 ppt). The most extreme salinities were measured at Site 4 (Site 4: FW and 28 ppt; Site 1: 5 and 26 ppt; Table 5-2). Although temperature was obviously linked to season, salinity did not appear to be affected by season.

Table 5-2. Temperature and salinity (daily mean and range) measured at two sites of Cedar Key salt marsh.

Date	Morning Tide	Site 1 Temperature (°C)	Site 4 Temperature (°C)	Site 1 Salinity (ppt)	Site 4 Salinity (ppt)
1/17/95	Low	14.2 13.5-14.5	13.7 11.2-15.4	7 6.2 – 8	1.34 0-2
2/7/95	High	15 11-18	15.2 11.5-18.5	11 10-15	11.2 10-14
4/1/95	Low	21.6 17-24	21.3 17.2-24.5	15.8 8 – 25	17 10-25
4/4/95	High	21 18.5-25	21.6 18.2-25	8.8 6 – 15	14.2 12-20
7/25/95	Low	30.1 27.7-32.8	30.1 27.7-32.8	20.2 12-24.5	18.8 12-23
8/5/95	High	30.8 24.1-35	27.4 17.5-34	14.56 11.3-19.5	14.2 4.5-22
10/17/95	High	22.6 18-26.5	22.08 17.5-25.4	6.2 5-7	1.2 FW-2
10/18/95	Low	26.4 24.2-29	26.7 23.9-30	13 10 – 22	9.8 5-24
12/16/95	High	20.6 18-22	20.6 17-24	18 15 – 26	23.6 19-28
1/6/96	Low	14.4 11-17.9	13.8 9-18	7.8 5-10	4 FW-8
5/9/96	High	29.4 24.5-34	29.8 25-34	21.2 16-25	22.4 19-26
5/16/96	Low	27.9 24-33	28.3 24-33	23.9 20.5-26	23.3 19.5-26
9/6/96	High	29.4 26-34	29.4 26-33	20.7 16-24	14 5.5-24

Discussion

The pattern of dissolved oxygen availability in the Cedar Key salt marsh system appears to be strongly influenced by photosynthetic processes as well as tidal conditions. Hypoxic conditions ($<2.0 \text{ mg l}^{-1} \text{ O}_2$) were not infrequent events, occurring on 11 of the 13 dates measurements were taken. Extreme hypoxia ($<1.0 \text{ mg l}^{-1} \text{ O}_2$) was recorded on 6 of the 13 dates. Surprisingly, extreme hypoxia was also documented at Cedar Key sites under colder conditions, when oxygen solubility is higher (2/7/95, Tables 5-1, 5-2). When hypoxia occurred, it was usually temporally associated with low tide and isolation from the surrounding system regardless of time of day. During isolation at low tide microbial metabolism of the heavy organic bottom sediments may contribute to hypoxic episodes (Belanger, 1981). Other environmental factors, such as freshwater runoff, current, and wind are also likely to influence aquatic oxygen levels and may account for the high degree of temporal and spatial variance in the patterns of hypoxia documented.

Although local generation of hypoxic conditions through pool isolation and/or nocturnal photosynthetic oxygen consumption appear to be factors affecting hypoxia in the Cedar Key system, this is not the case in other coastal systems. Seasonal, severe hypoxia ($<2.0 \text{ mg l}^{-1} \text{ O}_2$) occurs in the bottom waters of the Northern Gulf of Mexico. The temporal and spatial variability in hypoxic water masses in this region is correlated with the amplitude and phasing of freshwater discharge from the Mississippi River (Justic et al., 1993; Rabalais et al., 1998). Anthropogenic nutrient loads (e.g. Synthetic fertilizers, detergents) appear to overwhelm homeostatic mechanisms of the marine coast ecosystem to produce hypoxic water masses below the pycnocline of the Louisiana shelf (Justic et

al., 1993; Rabalais et al., 1998). Strong variation in oxygen levels has also been reported in Tampa Bay, ranging from 1.4-11.6 mg l⁻¹ (Simon, 1974). These hypoxic episodes were reportedly attributed to anthropogenically induced factors, such as pollution sources, or resuspension of anaerobic bottom sediments from ship's propellers (Simon, 1974).

Hypoxia is also a common occurrence in the near-shore, shallow waters of the Chesapeake Bay (Carter et al., 1978; Tyler, 1984; Seliger et al., 1985; Breitburg, 1990). Breitburg (1990) measured oxygen concentration, salinity, and temperature in the shallow inland waters of the Chesapeake Bay over two summers. In the Chesapeake Bay, dissolved oxygen concentrations dipped below 2 mg l⁻¹ on 45% and below 1 mg l⁻¹ on 9% of summer days measured. Minimum daily DO concentrations ranged from 0.47 to 7.16 mg l⁻¹, with the lowest minima occurring during the dark and morning hours. There was a diel pattern to oxygen concentration, with photosynthetic oxygen production increasing DO to levels tolerable to most estuarine organisms even on days with near anoxic daily minima (3.4 mg l⁻¹, Breitburg, 1990). In the Chesapeake Bay, hypoxia appears to be caused by stratification and an influx of oxygen-depleted lower layers into the shallows of the shoreline. The lower layers of the stratified deep waters of the Chesapeake Bay area are depleted of oxygen by microbial degradation of organic matter. Wind and tidal currents force intrusions of these hypoxic waters into the shallow inshore areas of the Bay (Officer et al., 1984; Breitburg, 1990).

Temperatures recorded in this study were similar to those of other north Florida salt marshes (Cedar Key: 9-33 °C, this study; 15-31 °C, Haney, 1995; St. Marks and Wakulla marshes, 12-36 °C, Subrahmanyam and Drake, 1975; Florida coastal marshes: 12.5-37.5 °C, Kilby, 1955). Kilby's (1955) measurements of several North Florida

coastal marshes revealed an extensive salinity range in the inner tide pools (1.2-35.6 ppt) although he surmised that there was generally low fresh water influx in these systems. The current study found that daily salinity minima ≤ 5 ppt were common, occurring on five of the 13 dates recorded, and daily means of ≤ 5 ppt occurred on three occasions. However, the average mean daily salinity for the sites was approximately 14 ppt, in agreement with Kilby (1955). Seasonal salinity increases have been reported in other North Florida salt marshes and attributed to different rates of evaporation (e.g., 2 ppt, February, 32 ppt June, Subrahmanyam and Drake, 1975). Such seasonal effects would be obscured by rainfall events, and no seasonal relationship between salinity and tide was evident in this data set.

A feature of coastal marshes is variation in species composition with elevation. It is generally accepted that the upper inland range limit (inland) of aquatic species within these coastal habitats is determined by physiological tolerance to extremes in environmental conditions, such as salinity and temperature, while their seaward range is limited by competitive interactions (Adam, 1990). As such, there has been much effort toward quantifying the physiological tolerance of inland aquatic species in these systems. In the interior waters of the salt marsh, where the environmental measurements were taken (Figure 5-1), the ichthyofauna is dominated by the sailfin molly, *Poecilia latipinna* (Kilby, 1955; pers. obs.), with diamond killifish (*Adinia xenica*), longnose killifish (*Fundulus similis*), Gulf killifish, (*Fundulus grandis*), and southern sheepshead minnow (*Cyprinodon variegatus*) also present in great numbers. Many of these species have been studied for their euryhaline characteristics (Table 5-3).

Table 5-3. Summary of salinity tolerances of species commonly occurring at the Cedar Key sites where measurements were taken.

Species	Salinity Tolerance	Reference
<i>Adinia xenica</i>	0.5-95+ ppt	Nordlie, 1987
<i>Cyprinodon variegatus</i>	FW-142 ppt	Simpson and Gunter, 1956; Kushlan, 1980
<i>Fundulus confluentus</i>	FW-100 ppt	Nordlie, 2000
<i>Fundulus grandis</i>	FW-110 ppt	Nordlie, 2000
<i>Fundulus similis</i>	FW-100 ppt	Nordlie, 2000
<i>Mugil cephalus</i>	FW-54 ppt	Nordlie and Leffler, 1975
<i>Poecilia latipinna</i>	FW-70 to 80 ppt	Nordlie et al. 1992

Since oxygen availability can critically affect species habitat use (Roberts, 1975; Kramer, 1983a, 1987; Chapman and Liem, 1995), those fish commonly found in the protected inland areas of the mudflat are likely to share not only tolerance of euryhaline conditions, but also tolerance to periodic extreme hypoxia. Surprisingly little has been published on aquatic hypoxia as a factor for survival in tidal marsh environments. Those fish commonly occurring in the inland waters of Cedar Key probably use ASR to survive these periods of hypoxia. However, since ASR in some circumstances increases exposure to aerial predation (Kramer et al. 1983), salt marsh fishes are likely to have developed other behavioral, or physiological, mechanisms to minimize their time at the surface. Sailfin mollies from the Cedar Key salt marsh use ASR in response to hypoxia; however, the threshold is extremely low (Chapter 3). In addition, use of ASR as an immediate response to hypoxia in the laboratory declined in response to acclimation to hypoxia, suggesting more gradual physiological compensation that decreased the threshold for ASR (Chapter 3). Behavioral and physiological mechanisms in sailfin mollies from the Cedar Key salt marsh appear to provide sufficient compensation to mitigate mortality and permit continued reproduction under extreme hypoxia.

Dissolved oxygen concentration was measured in the upper 10 cm of the water column. It should be noted that hypoxic bottom water commonly occurs in many estuarine and coastal systems, and, had this been measured, overall hypoxia would have been greater. Sediment oxygen levels are typically anoxic in coastal and estuarine systems, and many studies of adaptations to hypoxia (such as anaerobic capacity, reduced metabolic rate [VO_2], or critical tension [P_c]) have focused on benthic or burrowing fishes. For example, *Helcogramma medium*, a pacific benthic triplefin blenny of rocky

mid-tidal, can often be found under rocks and exposed to air for periods up to four h. In air, oxygen uptake occurs across gills, buccopharyngeal epithelium, and skin. During periods of air exposure, individuals of this species exhibit a 40% reduction in VO_2 compared to their aquatic rate, as well as an accumulation of blood lactate, indicating that metabolic requirements are supplemented by anaerobic metabolism (Innes and Wells, 1985). An extreme example of a burrowing species is *Ammodytes hexapterus*, a sand eel that burrows into intertidal or subtidal sediments of the Pacific for up to 6 months during the winter. During other times of the year it is pelagic, burrowing only to avoid predation or conserve energy. Producing an unstable burrow opening, the coarse sand in which it lives often collapses. When buried, without significant circulation with the water column, the sand eel's oxygen uptake depends upon ventilating its gills with hypoxic interstitial waters. These fish show a seasonal reduction in VO_2 (at both 5° and 12° C, winter acclimatized fish exhibited a 17% reduction compared to summer acclimatized fish) and P_c (P_c winter=16 Torr; P_c summer=30 Torr; Quinn and Schneider, 1991).

In summary, the temporal and spatial patterns of hypoxia in coastal salt marshes like the Cedar Key salt marsh are extremely complex reflecting the complex interactions of tide, photosynthesis, and water input, as well as local habitat characteristics that impact mixing and rates of production and respiration. Despite the number of interactive variables influencing dissolved oxygen availability, there were clear seasonal and diurnal trends. The latter seem to be strongly affected by tidal cycles. Water-breathing organisms that survive in these systems throughout the year must show either strong diel migrations or very high tolerance to fluctuating physico-chemical conditions.

CHAPTER 6

SUMMARY AND CONCLUSIONS

A central issue in physiological ecology is an understanding of how animals respond physiologically to natural variation in the physical environment, how this influences the distribution and abundance of populations, and the evolutionary consequences of such responses. For fishes, availability of dissolved oxygen is one abiotic factor that exerts a strong selective force in aquatic systems, affecting habitat quality, growth, survival and reproduction. Although much effort has been directed towards describing the physiological, behavioral, and morphological adaptations to deoxygenation in fishes, little is known about their interaction and the potential evolutionary consequences of hypoxia adaptation. In this dissertation, I used an integrative approach to address these issues by combining studies of behavioral, morphological, and physiological response to hypoxia both within and between populations. This study was designed to understand mechanisms compensating for hypoxia in the sailfin molly, *Poecilia latipinna*, a live-bearing poeciliid fish, with a focus on behavioral and physiological response, the fitness consequences of hypoxia adaptation, and implications of hypoxia for interdemographic variation. The primary focus of this study was on *Poecilia latipinna* from the salt marsh of Cedar Key, Florida.

Primary conclusions from this study:

- 1) In the mudflats of the Cedar Key salt marsh, dissolved oxygen levels are highly variable, conforming to the general trend of coastal salt marshes. Hypoxic conditions ($<2.0 \text{ mg l}^{-1}$) were frequent events at the collection site, occurring on 11 of the 13 sampling dates over a 21-m period. Despite the number of interactive variables that influenced dissolved oxygen availability, there were clear diurnal trends, with dissolved oxygen levels being lower near sunrise. In addition, oxygen levels seem to be strongly affected by season and tidal cycles. Water-breathing organisms that survive in these systems throughout the year must show either strong diel migrations or very high tolerance to fluctuating physico-chemical conditions (e.g., *Poecilia latipinna*).
- 2) The mass-corrected routine metabolic rate of female *P. latipinna* carrying late stage embryos was 35% higher than that of males and 23% higher than that of females carrying fertilized ova or early-stage embryos. In addition, gestating females exposed to extreme hypoxia spent approximately 20% more time conducting aquatic surface respiration (surface skimming behavior, ASR) than non-gestating females, further supporting a brood-related increase in oxygen demand. This increase in time spent on ASR could directly increase maternal predation risk in hypoxic waters.
- 3) *Poecilia latipinna* acclimated to chronic extreme hypoxia (6 wk at $1.0 \text{ mg l}^{-1} \text{ O}_2$) exhibited higher hemoglobin [Hb] and red blood cell (RBC) concentrations, and a 15-20% lower critical oxygen tension (P_c) than fish acclimated to normoxia. This conforms to

several other studies representing a phylogenetically diverse suite of taxa and ecological groupings. However, sailfin mollies exhibited lower values of [Hb] and Hct relative to a standard curve for freshwater fishes, which may reflect low energetic demands in this species. The routine total metabolic rate (RMR) of *P. latipinna* measured in this study falls below Winberg's (1961) standard curve for fishes and also below the standard curve for cyprinodontiforms.

4) Gill ventilation rates increased twofold under acclimation to extreme hypoxia (six wk at $1.0 \text{ mg l}^{-1} \text{ O}_2$), a response that did not diminish with time. However, the use of aquatic surface respiration (ASR) was an immediate response to hypoxia that decreased over the acclimation period, suggesting that physiological compensation reduces the ASR threshold. Aquatic surface respiration is more efficient than simply increasing ventilation and can occur as an immediate response to hypoxia; however, the costs of ASR (energetic cost, time at the surface, predation risk) may account for the strong decline with physiological acclimation.

5) There were no significant effects of hypoxia on mortality when fish were acclimated to extreme hypoxia for six wk, but effects on condition varied with gender. Males showed a significant diminution of condition after six wk of chronic hypoxia. There was no significant reduction in condition of females and no effect of hypoxia treatment on the number of gestating females. It is proposed that plastic behavioral and physiological responses in *P. latipinna* compensate for extreme hypoxia to a degree that mitigates reduced condition in females and a decrease in survivorship in both genders, and continued

reproduction in a laboratory setting. However, there may be predation costs in the field related to ASR, particularly for gestating females.

6) Comparisons of respiratory characters (physiological, morphological, and behavioral) between *P. latipinna* from a periodically-hypoxic habitat and a well-oxygenated habitat suggest that strong selection pressure for hypoxia tolerance in oxygen-scarce habitats may lead to interdemic variation among populations with broad habitat ranges. Under acclimation to chronic hypoxia (six wk at $1.0 \text{ mg l}^{-1} \text{ O}_2$), *P. latipinna* from a periodically hypoxic environment (Cedar Key salt marsh) spent less time conducting ASR, had lower gill ventilation rates, and spent less time near the surface than conspecifics from the well-oxygenated waters of the Santa Fe River. Hypoxia acclimation reduced the critical oxygen tension in both populations, but population level differences were maintained. Cedar Key fish had a 14% greater gill surface area relative to their body size than Santa Fe River fish, perhaps, in part, accounting for their greater tolerance to hypoxia. The source of these interdemic differences remains to be determined and will be important to understanding of the role of hypoxic waters in the maintenance and promotion of physiological diversity.

LIST OF REFERENCES

- Adam, P. 1990. Saltmarsh Ecology. Cambridge University Press. Cambridge, MA. 461 p.
- Aota, S., K.D. Holmgren, P. Gallagher, and D.J. Randall. 1990. A possible role for catecholamines in the ventilatory responses associated with internal acidosis or external hypoxia in rainbow trout, *Oncorhynchus mykiss*. Journal of Experimental Biology **151**:57-70.
- Arthington, A.H. and L.N. Lloyd. 1989. Introduced poeciliids in Australia and New Zealand. Pages 333-347 in G.K. Meffe and F.F. Snelson, Jr., editors. Ecology and evolution of livebearing fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, New Jersey. 453 p.
- Baird, R.C. 1968. Aggressive behavior and social organization in *Mollienesia latipinna* (LeSueur). Texas Journal of Science **20**:157-176.
- Baird, R.C. 1974. Aspects of social behavior of *Poecilia latipinna* (Lesueur). Reviews in Biology of the Tropics **21**:399-416.
- Balsano, J.S., E.M. Rasch, and P.J. Monaco. 1989. The evolutionary ecology of *Poecilia formosa* and its triploid associate. Pages 277-297 in G.K. Meffe and F.F. Snelson, Jr., editors. Ecology and evolution of livebearing fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, New Jersey. 453 p.
- Bartlett, G.R. 1978. Water soluble phosphates of fish red blood cells. Canadian Journal of Zoology **56**:870-877.
- Beadle, L.C. 1981. The Inland Waters of Africa. An Introduction to Tropical Limnology. Longman, London. 475 p.
- Beamish, F.W.H. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. III. Influence of oxygen. Canadian Journal of Zoology **42**:355-366.
- Beauchat, C.A. and D. Vleck. 1990. Metabolic consequences of viviparity in a lizard, *Sceloporus jarrovi*. Physiological Zoology **63**:555-570.
- Bejda, A.J., B.A. Phelan, and A.L. Studholme. 1992. The effect of dissolved oxygen on the growth of young-of-the-year winter flounder, *Pseudopleuronectes americanus*. Environmental Biology of Fishes **34**:321-327.

- Belanger, T.V. 1981. Comparison of benthic oxygen demand measurement techniques. *Environmental Sciences* **44**:204-213.
- Bel'Chenko, L.A. and O.V. Kel. 1992. Adaptation to hypoxia in crucian carp, *Carassius carassius* and wild goldfish, *Carassius auratus gibelio*. *Journal of Ichthyology* **32**:114-124.
- Bel'Chenko, L.A. and O.V. Kel. 1992. Adaptation to hypoxia in crucian carp, *Carassius carassius* and wild goldfish, *Carassius auratus gibelio*. *Journal of Ichthyology* **32**:114-124.
- Berglund, A., G. Rosenqvist, and I. Svensson. 1986. Reversed sex roles and parental energy investment in zygotes of two fish (Syngnathidae) species. *Marine Ecology Progress Series* **29**:209-214.
- Bindon, S.D., J.C. Fenwick, and S.F. Perry. 1994. Branchial chloride cell proliferation in the rainbow trout, *Oncorhynchus mykiss*: Implications for gas transfer. *Canadian Journal of Zoology* **72**:1395-1402.
- Birchard, G.F., C.P. Black, G.W. Schuett, and V. Black. 1984. Influence of pregnancy on oxygen consumption, heart rate and hematology in the garter snake: implications for "the cost of reproduction" in live bearing reptiles. *Comparative Biochemistry and Physiology A* **77**: 519-523.
- Blackburn, D.G., H.E. Evans, and L.J. Vitt. 1985. The evolution of fetal nutritional adaptations. Pages 437-439 in R. Duncker and G.M. Fleischer, editors. *Vertebrate Morphology*. Gustav Fischer Verlag, New York. 179 p.
- Blaxhall, P.C. 1972. The haematological assessment of the health of freshwater fish. A review of selected literature. *Journal of Fish Biology* **4**:593-604.
- Blaxhall, P.C. and K.W. Daisy. 1973. Routine haematological methods for use with fish blood. *Journal of Fish Biology* **5**:771-781.
- Blazka, P. 1958. The anaerobic metabolism of fish. *Physiological Zoology* **31**:117-128.
- Boehlert, G.W., M. Kusakari, M. Shimizu, and J. Yamada. 1986. Energetics during embryonic development in kurosoi, *Sebastes schlegeli*. *Journal of Experimental Marine Biology and Ecology* **101**:239-256.
- Boehlert, G.W., M. Kusakari, and J. Yamada. 1991. Oxygen consumption of gestating female *Sebastes schlegeli*: estimating the reproductive costs of livebearing. *Environmental Biology of Fishes* **30**: 81-89.

- Boehlert, G.W. and M.M. Yoklavich. 1984. Reproduction, embryonic energetics, and the maternal-fetal relationship in the viviparous genus *Sebastes* (Pisces: Scorpaenidae). *Biological Bulletin* **167**:354-370.
- Boese, B.L. 1988. Hypoxia-induced respiratory changes in English sole (*Parophrys vetulus* Girard). *Comparative Biochemistry and Physiology A* **89**:257-260.
- Bonnetto, A., W. Dioni, and C. Pignalberi. 1969. Limnological investigations of biotic communities in the Middle Parana River Valley. *Verhandlungen Internationale Vereinigung Limnologie* **17**:1035-1050.
- Borowsky, R.L. 1987. Genetic polymorphism in adult male size in *Xiphophorus variatus* (Atheriniformes: Poeciliidae). *Copeia* **1987**:782-787.
- Boutilier, R.G., G. Dobson, U. Hoeger, and D.J. Randall. 1988. Acute exposure to graded levels of hypoxia in rainbow trout (*Salmo gairdneri*): metabolic and respiratory adaptations. *Respiration Physiology* **71**:69-82.
- Brauner, C. and D.J. Randall. 1998. Interactions between ion and gas transfer in freshwater teleost fish. *Comparative Biochemistry and Physiology A* **119**:3-8.
- Breitburg, D.L. 1990. Near-shore hypoxia in the Chesapeake Bay: patterns and relationships among physical factors. *Estuarine, Coastal and Shelf Sciences* **30**:593-609.
- Breitburg, D.L. 1994. Behavioral response of fish larvae to low dissolved oxygen concentration in a stratified water column. *Marine Biology* **120**:615-625.
- Bridges, D.W., J.J. Jr. Cech, and D.N. Pedro. 1976. Seasonal hematological changes in winter flounder *Pseudopleuronectes americanus*. *Transactions of the American Fisheries Society* **105**:596-600.
- Brown, B.A. 1993. *Hematological Principals and Procedures*. Lea and Febiger, Philadelphia, PA. 453 p.
- Burd, B.J. 1988. Comparative gill characteristics of *Munida quadrispina* (Decapoda, Galatheididae) from differing habitat oxygen conditions. *Canadian Journal of Zoology* **66**:2320-2333.
- Burgess, G.H. 1980. *Poecilia latipinna* (Lesueur), Page 549 in D.S. Lee, C.R. Gilbert, C.H. Hocutt, R.E. Jenkins, D.E. McAllister, and J.R. Stauffer, Jr. editors. *Atlas of North American freshwater fishes*. Pub. No. 1980 12 North Carolina Biological Survey, North Carolina State Museum of Natural History, Raleigh, NC. 854 p.

- Burggren, W.W. 1982. "Air gulping" improves blood oxygen transport during aquatic hypoxia in the goldfish, *Carassius auratus*. *Physiological Zoology* **55**:327-334.
- Burton, D.T. and A.G. Heath. 1980. Ambient oxygen tension (PO₂) and transition to anaerobic metabolism in three species of freshwater fish. *Canadian Journal of Fisheries and Aquatic Sciences* **37**:1216-1224.
- Cambell, T. and F. Murru. 1990. An Introduction to Fish Hematology. *Compendium of Continuing Education for Practicing Veterinarian* **12**:525-532.
- Cameron, J.N. 1989. Metabolism and energetics. Pages 23-35 *in* J.N. Cameron, editor. *The Respiratory Physiology of Animals*. Oxford University Press, New York. 353 p.
- Carter, G.S. 1934. Results of the Cambridge Expedition to British Guiana, 1933. The freshwaters of the rain-forest areas of British Guiana. *Journal of the Linnean Society, Zoology* **39**:147-193.
- Carter, G.S. 1955. *The Papyrus Swamps of Uganda*. Cambridge, Heffner, 25 p.
- Carter, G.S. and L.C. Beadle. 1931. The fauna of the swamps of the Paraguayan Chaco in relation to its environment. II. Respiratory adaptations in the fishes. *Journal of the Linnean Society, Zoology* **37**:327-368.
- Carter, H.H., R.J. Regier, E.W. Schiemer, and J.A. Michael. 1978. The summer time vertical distribution of dissolved oxygen at the Calvert Cliffs generating station: a physical interpretation. Chesapeake Bay Institute, Johns Hopkins University, Special Report 60.
- Cech, J.J. Jr., C.G. Campagna, and S.J. Mitchell. 1979. Respiratory responses of largemouth bass (*Micropterus salmoides*) to environmental changes in temperature and dissolved oxygen. *Transactions of the American Fisheries Society* **108**:166-171.
- Cech, J.J. Jr., M.J. Massingill, B. Vondracek, and A.L. Linden. 1985. Respiratory metabolism of mosquitofish, *Gambusia affinis*: effects of temperature, dissolved oxygen and sex difference. *Environmental Biology of Fishes* **13**:297-307.
- Chapman. L.J. 1995. Seasonal dynamics of habitat use by an air-breathing catfish (*Clarias liocephalus*) in a papyrus swamp. *Ecology of Freshwater Fish* **4**:113-123.
- Chapman, L.J. and C.A. Chapman. 1998. Hypoxia tolerance of the mormyrid *Petrocephalus catostoma*: implications for persistence in swamp refugia. *Copeia* **1998**:762-768.

- Chapman L.J. and C.A. Chapman. 1993. Fish populations in tropical floodplain pools: A re-evaluation of Holden's data on the River Sokoto. *Ecology of Freshwater Fish* **2**:23-30.
- Chapman, L.J., C.A. Chapman, D.A. Brazeau, B. McLaughlin, and M. Jordan. 1999. Papyrus swamps, hypoxia, and faunal diversification: variation among populations of *Barbus neumayeri*. *Journal of Fish Biology* **54**:310-327.
- Chapman, L.J., C.A. Chapman, and T.L. Crisman. 1998. Limnological observations of a papyrus swamp in Uganda: Implications for fish faunal structure and diversity. *Verhandlungen Internationale Vereinigung Limnologie* **26**:1821-1826.
- Chapman L.J., F. Galis, and J. Shinn. 2000. Phenotypic plasticity and the possible role of genetic assimilation: Hypoxia-induced trade-offs in the morphological traits of an African cichlid. *Ecological Letters* **3**:387-393.
- Chapman, L.J. and K. Hulen. Implications of hypoxia for the brain size and gill surface area of mormyrid fishes. *Journal of Zoology* (In Press).
- Chapman, L.J., L.S. Kaufman, and C.A. Chapman. 1994. Why swim upside down?: a comparative study of two mochokid catfishes. *Copeia* **1994**:130-135.
- Chapman, L.J., L.S. Kaufman, C.A. Chapman, and F.E. McKenzie. 1995. Hypoxia tolerance in twelve species of East African cichlids: potential for low oxygen refugia in Lake Victoria. *Conservation Biology* **9**:1274-1288.
- Chapman, L.J. and D.L. Kramer 1991a. Limnological observations of an intermittent tropical dry forest stream. *Hydrobiologia* **226**:153-166.
- Chapman, L.J. and D.L. Kramer. 1991b. The consequences of flooding for the dispersal and fate of poeciliid fish in an intermittent tropical stream. *Oecologia* **87**:299-306.
- Chapman, L.J. and K.F. Liem. 1995. Papyrus swamps and the respiratory ecology of *Barbus neumayeri*. *Environmental Biology of Fishes* **44**:183-197.
- Clasen, J. 1998. Efficiency control of particle removal by rapid sand filters in treatment plants fed with reservoir water: a survey of different methods. *Water Science and Technology* **37**:19-26.
- Coburn, C.B., Jr. 1973. Red blood cell hematology of fishes: a critique of techniques and a compilation of published data. *Journal of Marine Science* **2**:37-58.

- Congleton, J.L. 1980. Observations on the responses of some southern California tidepool fishes to nocturnal hypoxic stress. *Comparative Biochemistry and Physiology A* **66**:719-722.
- Constantz, G.D. 1989. Reproductive Biology of Poeciliid Fishes. Pages 33-50 *in* G.K. Meffe and F.F. Snelson, editors. *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, New Jersey. 453 p.
- Courtenay, W. R. Jr. and G.K. Meffe. 1989. Small Fish in Strange Places: a Review of Introduced Poeciliids. Pages 319-331 *in* G.K. Meffe and F.F. Snelson, editors. *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, New Jersey. 453 p.
- Crisman, T.L., L.J. Chapman and C.A. Chapman. 1998. Predictors of seasonal oxygen levels in small Florida lakes: the importance of color. *Hydrobiologia* **368**:149-155
- Cutwa, M.M. and R.G. Turingan. 2000. Intralocality variation in feeding biomechanics and prey use in *Archosargus probatocephalus* (Teleostei, Sparidae), with implications for the ecomorphology of fishes. *Environmental Biology of Fishes* **59**:191-198.
- Datta-Munshi, J.S. and G.M. Hughes. 1992a. Accessory Respiratory Organs. Pages 73-97 *in* A.A. Balkema, editor. *Air-breathing Fishes of India: their Structure, Function and Life History*. Academic Press, London. 338 p.
- Datta-Munshi, J.S. and G.M. Hughes. 1992b. Airbreathing Teleosts. Pages 23-30 *in* A.A. Balkema, editor. *Air-breathing Fishes of India: their Structure, Function and Life history*. Academic Press, London. 338 p.
- Davie, P.S. 1990. *Pacific Marlins: Anatomy and Physiology*. Massey University, Palmerston North, New Zealand. 87 p.
- Davis, J.C. and J.N. Cameron. 1970. Water flow and gas exchange at the gills of the rainbow trout, *Salmo gairdneri*. *Journal of the Fisheries Research Board Canada* **30**: 99-104.
- Day, T., J. Pritchard, and D. Schluter. 1994. A comparison of two sticklebacks. *Evolution* **48**:1723-1734.
- Dejours, P. 1994. Environmental factors as determinants in bimodal breathing: an introductory overview. *American Zoology* **34**:178-183.

- DeMarco, V. 1993. Metabolic rates of female viviparous lizards (*Sceloporus jarrovi*) throughout the reproductive cycle: do pregnant lizards adhere to standard allometry? *Physiological Zoology* **66**:166-180.
- DeSilva, C.D., S. Premawansa, and C.N. Keembiyahetty. 1986. Oxygen consumption in *Oreochromis niloticus* (L.) in relation to development, salinity, temperature and time of day. *Journal of Fish Biology* **29**:267-277.
- DeSilva, C.D. and P. Tytler. 1973. The influence of reduced environmental oxygen on the metabolism and survival of herring and plaice larvae. *Netherlands Journal of Sea Research* **7**:345-362.
- Dethlefsen, V. and H. von Westernhagen. 1983. Oxygen deficiency and effects on bottom fauna in eastern German Bight 1982. *Meeresforschung* **30**:42-53.
- Diamond, S.A., M.C. Newman, M. Mulvey, and S.I. Guttman. 1991. Allozyme genotype and time-to-death of mosquitofish, *Gambusia holbrooki*, during acute inorganic mercury exposure: A comparison of populations. *Aquatic Toxicology* (Amsterdam) **21**:119-134.
- Diaz, R.J., R.J. Neubauer, L.C. Schaffner, L. Pihl, and S.P. Baden. 1992. Continuous monitoring of dissolved oxygen in an estuary experiencing periodic hypoxia and the effect of hypoxia on macrobenthos and fish Pages 1055-1068 *in* Marine coastal eutrophication: the response of marine transitional systems to human impact, problems and perspectives for restoration: proceedings of an international conference. Bologne, Italy. 21-24 March 1990 Amsterdam: New York.
- Di Prisco, G. and M. Tamburrini. 1992. The hemoglobins of marine and freshwater fish: the search for correlations with physiological adaptations. *Comparative Biochemistry and Physiology B* **102**:661-671.
- Dygert, P.H. and D.R. Gunderson. 1991. Energy utilization by embryos during gestation in viviparous copper rockfish, *Sebastes caurinus*. *Environmental Biology of Fishes* **30**:165-171.
- Eckert, R. and D. Randall. 1983. *Animal Physiology, Mechanisms and Adaptations*. W.H. Freeman and Co. New York. 830 p.
- Endler, J.A. 1980. Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34**:76-91.
- Evans, D.H. 1975. The effects of various external cations and sodium transport inhibitors on sodium uptake by the sailfin molly, *Poecilia latipinna*, acclimated to sea water. *Journal of Comparative Physiology* **96**:111-115.

- Evans, D.H. 1987. The fish gill: Site of action and model for toxic effects of environmental pollutants. *Environmental Health Perspectives* **71**:47-78.
- Evans, D.H. 1993. Osmotic and ionic regulation. Pages 315-341 in D.H. Evans editor. *The Physiology of Fishes*. Marine Science Series. CRC Press, Boca Raton, FL. 592 p.
- Farr, J.A. 1989. Sexual Selection and Secondary Sexual Differentiation in Poeciliids: Determinants of Male Mating Success and the Evolution of Female Mate Choice. Pages 91- 123 in G.K. Meffe and F. F. Snellson Jr., editors. *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, New Jersey 453 p.
- Farr, J.A. and J. Travis. 1989. The effect of ontogenetic experience on variation in growth and sexual behavior in the sailfin molly, *Poecilia latipinna* (Pisces: Poeciliidae). *Environmental Biology of Fishes* **26**:39-48.
- Farr, J.A. and J. Travis. 1986. Fertility advertisement by female sailfin mollies, *Poecilia latipinna* (Pisces: Poeciliidae). *Copeia* **1986**:467-472.
- Fernandes, M.N., W.R. Barrioneuvo, and F.T. Rantin. 1995. Effects of thermal stress on respiratory responses to hypoxia of a south American prochilodontid fish, *Prochilodus scrofa*. *Journal of Fish Biology* **46**:123-133.
- Fernandes, M.N., F.T. Rantin, A.L. Kalinin, and S.E. Moron. 1994. Comparative study of gill dimension of 3 erythrinid species in relation to their respiratory function. *Canadian Journal of Zoology* **72**:160-165.
- Fievet, B., G. Claireaux, S. Thomas, and R. Motais. 1988. Adaptive respiratory responses of trout to acute hypoxia. III. Ion movements and pH changes in the red blood cell. *Respiration Physiology* **74**:99-114.
- Foscarini, R. 1989. A comparative study of the skin and gill structure in oviparous and viviparous freshwater fish larvae. *Journal of Fish Biology* **34**:31-40.
- Frangioni, G., R. Berti, and G. Borgioli. 1997. Hepatic respiratory compensation and haematological changes in the cave cyprinid, *Phreatichthys andruzzii*. *Journal of Comparative Physiology B* **167**:461-467.
- Frische, R. and S. Nilsson. 1993. Cardiovascular and ventilatory control during hypoxia. Pages 180-205 in J.C. Rankin and F. B. Jensen editors. *Fish ecophysiology*. Chapman and Hall, London. 421 p.

- Fry, F.E.J. 1957. The Aquatic Respiration of Fish. Pages 1-63 in M.E. Brown, editor. The Physiology of Fishes. Academic Press Inc., New York. 447 p.
- Fyhn, U.E.H., Fyhn H.J., Davis, B.J., Powers, D.A., Fink, W.L. and R.L. Garlick. 1979. Hemoglobin heterogeneity in Amazonian fishes. Comparative Biochemistry and Physiology A **62**:39-66.
- Galis, F. and C.D.N. Barel. 1980. Comparative functional morphology of the gills of African lacustrine cichlidae (Pisces, Teleostei). Netherland Journal of Zoology **30**:392-430.
- Gallaughner, P. and A.P. Farrell. 1998. Hematocrit and Blood Oxygen Carrying Capacity. Pages 185-227 in S.F. Perry and B.L. Tufts, editors. Fish Physiology, Volume 17: Fish Respiration. Academic Press, London. .
- Gee, J.H. and P.A. Gee. 1991. Reactions of gobioid fishes to hypoxia: bouyancy control and aquatic surface respiration. Copeia **1991**:17-28.
- Gee, J.H., R.F. Tallman and H.J. Smart. 1978. Reactions of some Great Plains fishes to progressive hypoxia. Canadian Journal of Zoology **56**:1962-1966.
- Geevarghese, C. and P.A. John. 1984. Oxygen consumption of a gobiid fish, *Oligolepis acutipennis* (Cuv. and Val.) (Gobiidae, Teleostei). Comparative Physiology and Ecology **9**:290-294.
- Gessner, F. 1961. Der Sauerstoffhaushalt des Amazonas. Internationale Revue ges. Hydrobiologica **46**:542-561.
- Glass, M.L., Rantin, F.T., Verzola R.M.M., Fernandes M.N. and A.L. Kalinin. 1991. Cardiorespiratory synchronization and myocardial function in hypoxic carp, *Cyprinus carpio* L. Journal of Fish Biology **39**:143-149.
- Graham, J.B. 1997. Air-breathing Fishes: Evolution, Diversity, and Adaptation. Academic Press. San Diego, CA. 229 p.
- Graham, J.B. 1985. Seasonal and environmental effects on the blood hemoglobin concentrations of some Panamanian air-breathing fishes. Environmental Biology of Fishes **12**:291-301.
- Greaney, G.S., A.R. Place, R.E. Cashon, G. Smith, and D.A. Powers. 1980. Time course changes in enzyme activities and blood respiratory properties of killifish during long-term hypoxia acclimation. Physiological Zoology **53**:136-144.

- Greaney, G.S. and D.A. Powers. 1978. Allosteric modifiers of fish hemoglobins: *in vitro* and *in vivo* studies of the effect of ambient oxygen and pH on erythrocyte ATP concentrations. *Journal of Experimental Zoology* **203**:339-350.
- Grier, H.J. 1973. Reproduction in the teleost *Poecilia latipinna*: an ultrastructural and photoperiodic investigation. M.S. Thesis. University of South Florida, Tampa, FL. 180 p.
- Grier, H.J. 1980. The morphology of enclosed testicular tubules in *Poecilia latipinna*. *Transactions of the American Microscopy Society* **99**:268-276.
- Guillette, L.J. Jr. 1982. The effects of gravidity on the metabolism of the reproductively bimodal lizard, *Sceloporus aenus*. *Journal of experimental Zoology* **223**:33-36.
- Guillette, L.J. Jr. 1987. The Evolution of Viviparity in Fishes, Amphibians and Reptiles: an Endocrine Approach. Pages 523-562 in D.O. Norris and R.E. Jones, editors. *Hormones and Reproduction in Fishes, Amphibians, and Reptiles*. Plenum Publishing Co., New York. 632 p.
- Gustafson, D.L. 1981. The influence of salinity on plasma osmolality and routine oxygen consumption in the sailfin molly, *Poecilia latipinna* (LeSueur), from a freshwater and estuarine population. M.S. Thesis. University of Florida, Gainesville, FL. 131 p.
- Haney, D.C. 1995. Responses made by the salt marsh teleost *Cyprinodon variegatus* (Atherinomorpha: Cyprinodontidae) to life in a variable salinity environment. Ph.D. Dissertation, University of Florida, Gainesville FL. 123 p.
- Harper, D.E., Jr., L.D. McKinney, R.R. Salzer, and R.J. Case. 1981. The occurrence of hypoxic bottom water off the upper Texas coast and its effect on the benthic biota. *Contributions in Marine Science* **24**:53-79.
- Haynes, J.L. 1995. Standardized classification of poeciliid development for life-history studies. *Copeia* **1995**:147- 154.
- Hecky, R.E. 1993. The eutrophication of Lake Victoria. *Verhandlungen Internationale Vereinigung Limnologie* **25**:39-48.
- Hoar, W.S. 1969. Reproduction. Pages 1-72 in W.S. Hoar and D.J. Randall, editors. *Fish physiology*. Vol III. Academic Press, New York. 485 p.
- Hochachka, P.W. 1993. Surviving hypoxia: mechanisms of control and adaptation. CRC Press. Boca Raton, FL. 570 p.

- Hochachka, P.W. and G.N. Somero. 1984. Biochemical Adaptation: Basic Mechanisms and Strategies. Pages 3-14 in P.W. Hochachka, editor. Biochemical Adaptation. Princeton University Press, Princeton, NJ. 535 p.
- Holeton, G.F. 1979. Environmental Physiology of Fishes. M.A. Ali, editor. Plenum Press, New York. 723 p.
- Holeton, G.F. and D.J. Randall. 1967. The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. *Journal of Experimental Biology* **46**:317-327.
- Holliday, F.G.T., J.H.S. Blaxter, and R. Lasker. 1964. Oxygen uptake of developing eggs and larvae of the herring (*Clupea harengus*). *Journal of the Marine Biology Association U.K.* **44**:711-723.
- Holopainen, I.J., J. Aho, and M. Vornanen. 1997. Phenotypic plasticity and predator effects on morphology and physiology of crucian carp in nature and in the laboratory. *Journal of Fish Biology* **50**:781-798.
- Houde, E.D. and R.C. Scheckter. 1983. Oxygen uptake and comparative energetics among eggs and larvae of three subtropical marine fishes. *Marine Biology* **72**:283-293.
- Houston, A.H., N. Dobric, and R. Kahurananga. 1996. The nature of hematological response in fish. Studies on rainbow trout *Oncorhynchus mykiss* exposed to simulated winter, spring and summer conditions. *Fish Physiology and Biochemistry* **15**:339-347.
- Hueppop, K. 1986. Oxygen consumption of *Asyanax fasciatus* (Characidae: Pisces): A comparison of epigeal and hypogean populations. *Environmental Biology of Fishes* **17**:299-308.
- Hughes, G.M. 1973. Respiratory responses to hypoxia in fish. *American Zoologist* **13**:475-489.
- Hughes, G.M. 1981. Effects of Low Oxygen and Pollutants on the Respiratory Systems of Fish. Pages 121-146 in A.D. Pickering, editor. Stress and Fish. Academic Press, London. 367 p.
- Hughes, G.M. 1984. Respiratory Adaptations of Marine Fish in Relation to their Mode of Life under Different Environmental Conditions. Pages 98-113 in L. Bois, J. Zadunaisky and R. Giles, editors. Toxins, Drugs and Pollutants in Marine Mammals. Proceedings in Life Science. Springer –Verlag, Berlin. 193 p.

- Hughes, G.M. and Y. Kikuchi. 1984. Effects of *in vivo* and *in vitro* changes in PO₂ on the deformability of red blood cells of rainbow trout (*Salmo gairdneri* R.). *Journal of Experimental Biology* **111**:253-257.
- Hughes, G.M. & Morgan, M. (1973). The structure of fish gills in relation to their respiratory function. *Biological Review*. Cambridge Philosophical Society **48**:419-475.
- Innes, A.J. and R.M.G. Wells. 1985. Respiration and oxygen transport functions of the blood from an intertidal fish, *Helcogramma medium* (Tripterygiidae). *Environmental Biology of Fishes* **14**:213-226.
- Itazawa, Y. and T. Takedo. 1978. Gas exchange in the carp gills in normoxic and hypoxic conditions. *Respiration Physiology* **35**:263-269.
- Jensen, F.B. 1991. Multiple Strategies in Oxygen and Carbon Dioxide transport by Haemoglobin. Pages 55-78 in A.J. Woakes, M.K. Grieshaber, C.R. Bridges, editors. *Physiological Strategies for Gas Exchange and Metabolism*. Cambridge University Press, Cambridge, MA. 266 p.
- Jensen, F.B., M. Nikinmaa, and R.E. Weber. 1993. Environmental Perturbations of Oxygen Transport in Teleost Fishes: Causes, Consequences and Compensations. Pages 162-179 in J.C. Rankin and F.B. Jensen, editors. *Fish Ecophysiology*. Chapman and Hall, London. 421 p.
- Jobling, M. 1994. Respiration and Metabolism. Pages 121-145 in M. Jobling, editor. *Fish Bioenergetics*. Chapman and Hall, London. 309 p.
- Johansen, K. 1970. Air-breathing in Fishes. Pages 361-411 in W.S. Hoar and D.J. Randall, editors. *Fish Physiology*. Vol IV. Academic Press, New York.
- Johansen, K., C.P. Mangum, and G. Lykkeboe. 1978. Respiratory properties of the blood of Amazon fishes. *Canadian Journal of Zoology* **56**:898-906.
- Johansen, K. and R.E. Weber. 1976. On the Adaptability of Haemoglobin Function to Environmental Conditions. Pages 219-234 in P. Spencer Davies, editor. *Perspectives in Experimental Biology*. Vol. I. Zoology. Pergamon Press, New York.
- Johnston, I.A. 1975. Anaerobic metabolism in the carp, (*Carassius carassius* L.). *Comparative Biochemistry and Physiology B* **51**:235-241.
- Junk, W.J., G.M. Soares, and F.M. Carvalho. 1983. Distribution of fish species in a lake of the Amazon river floodplain near Manaus (Lago Camaleao), with special reference to extreme oxygen conditions. *Amazoniana* **7**:397-431.

- Justic, D., N.N. Rabalais, R.E. Turner and W.J. Wiseman. 1993. Seasonal coupling between riverborne nutrients, net productivity and hypoxia. *Marine Pollution Bulletin* **26**:184-189.
- Kallman, K.D. 1983. The sex determining mechanism of the poeciliid fish, *Xiphophorus montezumae*, and the genetic control of the sexual maturation process and adult size. *Copeia* **1983**:755-769.
- Kallman, K.D. 1984. A new look at sex determination in poeciliid fishes. Pages 95-171 in B.J. Turner, editor. *Evolutionary genetics of fishes*. Plenum Publishing Co., New York. 636 p.
- Kamler, E. 1992. *Early Life History of Fish: an Energetics Approach*. Chapman and Hall, New York. 267 p.
- Keenleyside, M.H.A. 1979. *Diversity and Adaptation in Fish Behavior*. Springer-Verlag, Berlin. 209 p.
- Kilby, J.D. 1955. The fishes of two Gulf coastal marsh areas of Florida. *Tulane Studies in Zoology* **2**:175-247.
- Kinkead, R. and S.F. Perry. 1991. The effects of catecholamines on ventilation in rainbow trout during hypoxia or hypercapnia. *Respiratory Physiology* **84**: 77-92.
- Kizito, Y.S., A. Nauwerck, L.J. Chapman, and M.J. Costa. 1993. A limnological survey of some Western Uganda crater lakes. *Limnologica* **23**:335-347.
- Kramer, D.L. 1983a. The evolutionary ecology of respiratory mode in fishes: an analysis based on the costs of breathing. *Environmental Biology of Fishes* **9**:145- 158.
- Kramer, D.L. 1983b. Aquatic surface respiration in the fishes of Panama: distribution in relation to risk of hypoxia. *Environmental Biology of Fishes* **8**:49-54.
- Kramer, D.L. 1987. Dissolved oxygen and fish behavior. *Environmental Biology of Fishes* **18**:81-92.
- Kramer, D.L., C.C. Lindsey, G.E.E. Moodie, and E.D. Stevens. 1978. The fishes and the aquatic environment of the central Amazon basin, with particular reference to respiratory patterns. *Canadian Journal of Zoology* **56**:717-729.
- Kramer, D.L., D. Manley, and R. Bougeois. 1983. The effects of respiratory mode and oxygen concentration on the risk of aerial predation in fishes. *Canadian Journal of Zoology* **61**:653-665.

- Kramer, D.L. and M. McClure. 1982. Aquatic surface respiration, a widespread adaptation to hypoxia in tropical freshwater fishes. *Environmental Biology of Fishes* **7**:47-55.
- Kramer, D.L. and J.P. Mehegan. 1981. Aquatic surface respiration, an adaptive response to hypoxia in the guppy, *Poecilia reticulata* (Pisces, Poeciliidae). *Environmental Biology of Fishes* **6**:299-313.
- Large, H.L. 1985. Life history tactics of the sailfin molly (*Poecilia latipinna*) in contrasting environments. M.S. Thesis, University of Central Florida, Orlando FL. 72 p.
- Lasker, R. and G.H. Theilacker, 1962. Oxygen consumption and osmoregulation by single pacific sardine eggs and larvae (*Sardinops caerulea* Girard). *Journal de Conseil* **27**:25-33
- Laurent, P and N. Hebibi. 1988. Gill morphometry and fish osmoregulation. *Canadian Journal of Zoology* **67**: 3055-3063.
- Laurent, P. and S.F. Perry. 1991. Environmental effects on fish gill morphology. *Physiological Zoology* **64**:4-25.
- Lee, D.S. C.R. Gilbert, C.H. Hocutt, R.E. Jenkins, D.E. McAllister and J.R. Stauffer, Jr. 1980. *Atlas of North American Freshwater Fishes*. North Carolina State Museum of Natural History, Raleigh, NC.
- Lewis, W.M. Jr. 1970. Morphological adaptation of cyprinodontoids for inhabiting oxygen deficient waters. *Copeia* **1970**: 319-326.
- Lewis, W.M. and F.H. Weibezahn. 1976. Chemistry, energy, and community structure in some Venezuelan fresh waters. *Arch. Hydrobiol. (suppl.)* **50**:45-207.
- Liem, K.F. 1980. Air Ventilation in Advanced Teleosts: Biomechanical and Evolutionary Aspects. Pages 57-91 *in*: M.A. Ali, editor. *Environmental Physiology of Fishes*. Plenum Press, New York.
- Luckner, C.L. 1979. Morphological and behavioral polymorphism in *Poecilia latipinna* males (Pisces: Poeciliidae). Ph.D. Dissertation, Louisiana State University, Baton Rouge LA. 156 p.
- Lumholt, J.P. and K. Johansen. 1979. Hypoxia acclimation in carp- how it affects O₂ uptake, ventilation, and O₂ extraction from water. *Physiological Zoology* **52**:38-49.

- Lykkeboe, G., K. Johansen, and G.M.O. Maloiy. 1975. Functional properties of hemoglobins in the teleost *Tilapia grahami*. *Journal of Comparative Physiology* **104**:1-11.
- Mackiernan, G. B. 1987. Dissolved oxygen in the Chesapeake Bay, processes and effects. *Proceedings of a seminar in hypoxic and related processes in Chesapeake Bay*. Maryland Sea Grant, College Park, Maryland. 177 p.
- Marinsky, C.A., A.H. Houston, and A. Murad. 1990. The effect of hemoglobin isomorph abundances in rainbow trout, *Salmo gairdneri*. *Canadian Journal of Zoology* **68**:884-888.
- Marvin, D.E. and A.C. Heath. 1968. Cardiac and respiratory responses to gradual hypoxia in 3 ecologically distinct species of fresh-water fish. *Comparative Biochemistry and Physiology A* **27**:349-355.
- McArdle, B.H. 1987. The structural relationship: regression in biology. *Canadian Journal of Zoology* **66**:2329-2339.
- McCorkle, F.M., J.E Chambers, and J.D. Yarbrough. 1979. Tolerance of low oxygen stress in insecticide-resistant and susceptible populations of mosquitofish (*Gambusia affinis*). *Life Sciences* **25**: 1513-1518.
- McKinsey, D.M. and L.J. Chapman. 1998. Dissolved oxygen and fish distribution in a Florida spring. *Environmental Biology of Fishes* **53**:211-223.
- McManus, M.G. and J. Travis. 1998. Effects of temperature and salinity on the life history of the sailfin molly (Pisces: Poeciliidae): lipid storage and reproductive allocation. *Oecologia* **114**: 317-325.
- Meffe, G.K. and F.F. Snelson, Jr. 1989. An Ecological Overview of Poeciliid Fishes. Pages 13-31 in G.K. Meffe and F.F. Snelson, Jr., editors. *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, New Jersey. 453 p.
- Milligan, C.L. and C.M. Wood. 1987. Regulation of blood oxygen transport and red cell pH, after exhaustive activity in rainbow trout (*Salmo gairdneri*) and Starry flounder (*Platichthys stellatus*). *Journal of Experimental Biology* **133**:263-282.
- Mittelbach, G.C., C.W. Osenberg, and P.C. Wainwright. 1999. Variation in feeding morphology between pumpkinseed populations: phenotypic plasticity or evolution? *Evolutionary Ecology Research* **1**:111-128.

- Morioka, Y. 1985. Growth, respiration and food requirement of flounder, *Paralichthys olivaceus*, in its early life history. Bulletin of Seikai Regional Fisheries Research Laboratory 62:67-77.
- Moser, H.G. 1967. Reproduction and development of *Sebastes paucispinis* and comparison of other rockfishes off California. Copeia 1967:773-779.
- Motta, P.J., S.F. Norton, and J.J. Luczkovich. 1995. Perspectives on the ecomorphology of bony fishes. Environmental Biology of Fishes 44:11-20.
- Muir and Hughes. 1969. Gill dimensions for 3 species of tunny. Journal of Experimental Biology 51:271-285.
- Murad, A., A.H. Houston, and L. Samson. 1990. Haematological response to reduced oxygen-carrying capacity, increased temperature and hypoxia in goldfish, *Carassius auratus* L. Journal of Fish Biology 36:289-305.
- Nacci, D., L. Coiro, D. Champlin, S. Jayaraman, R. McKinney, T.R. Gleason, W.R. Munns, J.L. Specker, and K.R. Cooper. 1999. Adaptations of wild populations of the estuarine fish *Fundulus heteroclitus* to persistent environmental contaminants. Marine Biology 134:9-17.
- Nelson, J.A., J.J. Magnuson, and W. Chulakasem. 1988. Blood oxygen capacity differences in yellow perch (*Perca flavescens*) from northern Wisconsin Lakes (USA) differing in pH. Canadian Journal of Fisheries and Aquatic Sciences 45:1699-1704.
- Nelson, J.A. and G.S. Mitchell. 1992. Blood chemistry response to acid exposure in yellow perch (*Perca flavescens*): Comparison of populations from naturally acidic and neutral environments. Physiological Zoology 65:493-514
- Nelson, J.A., Tang Y. and R.G. Boutilier. 1996. The effects of salinity change on the exercise performance of two Atlantic cod (*Gadus morhua*) populations inhabiting different environments. Journal of Experimental Biology 199:1295-1309.
- Nelson, J.A., Y. Tang, and R.G. Boutilier. 1994. Differences in exercise physiology between two atlantic cod (*Gadus morhua*) populations from different environments. Physiological Zoology 67:330-354.
- Nikinmaa, M. 1983. Adrenergic regulation of haemoglobin oxygen affinity in rainbow trout red cells. Journal of Comparative Physiology 152:67-72.
- Nikinmaa, M. and A. Salama. 1998. Oxygen Transport in Fish. Pages 141-170 in S.F. Perry and B.L. Tufts, editors. Fish Respiration. Academic Press, New York. 356 p.

- Nilsson, S. 1986. Control of Blood Flow. Pages 86-101 in S. Nilsson and S. Holmgren, editors. *Fish Physiology: Recent Advances*. Croom Helm, London. 198 p.
- Nordlie, F.G. 1985. Osmotic regulation in the sheepshead minnow *Cyprinodon variegatus* Lacepede. *Journal of Fish Biology* **26**:161-170.
- Nordlie, F.G. 1987. Salinity tolerance and osmotic regulation in the diamond killifish, *Adinia xenica*. *Environmental Biology of Fishes* **20**:229-232.
- Nordlie, F.G. 2000. Salinity responses in three species of *Fundulus* (Teleostei: Fundulidae) from Florida salt marshes. *Verhandlungen Internationale Vereinigung Limnologie* **27**:1-4.
- Nordlie, F.G., D.C. Haney, and S.J. Walsh. 1992. Comparisons of salinity tolerances and osmotic regulatory capabilities in populations of sailfin molly (*Poecilia latipinna*) from brackish and fresh waters. *Copeia* **1992**:741-746.
- Nordlie, F.G. and C.W. Leffler. 1975. Ionic regulation and the energetics of osmoregulation in *Mugil cephalus* Linneaus. *Comparative Biochemistry and Physiology A* **51**:125-131.
- Odum, H.T. and D.K. Caldwell. 1955. Fish respiration in the natural oxygen gradient of an anaerobic spring in Florida. *Copeia* **1955**:104-106.
- Odum, W.E. 1988. Comparative Ecology of Tidal Freshwater and Salt Marshes. Pages 147-176 in R.F. Johnston editor. *Annual Reviews in Ecological Systems*. Volume 19. Annual Reviews Inc. Palo Alto, California. 608 p.
- Officer, C.B., R.B. Biggs, J.L. Taft, L.E. Cronin, M.A. Tyler, and W.R. Boynton. 1984. Chesapeake Bay anoxia: origin, development, and significance. *Science* **223**:22-27.
- Oikari, K. and A. Soivino. 1975. Influence of sampling methods and anaesthetization on various haematological parameters of several teleosts. *Aquaculture* **6**:171-180.
- Oikawa, S. and Itazawa Y. 1985. Gill and body surface areas of the carp in relation to body mass with special reference to the metabolism-size relationship. *Journal of Experimental Biology* **117**:1-14.
- Oikawa, S. and Itazawa Y. 1992. Relationship between metabolic rate *in vivo* and body mass in a marine teleost, porgy, *Pagrus major*. *Fish Physiology and Biochemistry* **10**:177-182.

- Oikawa, S. and Y. Itazawa, 1993. Tissue respiration and relative growth of parts of body of a marine teleost, porgy *Pagrus major*, during early life stages with special reference to the metabolism-size relationship. *Comparative Biochemistry and Physiology A* **105**:741-744.
- Oikawa, S., Y. Itazawa, and M. Gotoh. 1991. Ontogenetic change in the relationship between metabolic rate and body mass in sea bream *Pagrus major* (Temminck and Schlegel). *Journal of Fish Biology* **38**:483-496.
- Olowo, J.P. and L.J. Chapman. 1996. Papyrus swamps and variation in the respiratory behavior of the African fish *Barbus neumayeri*. *African Journal of Ecology* **34**:211-222.
- Olson, K.R. 1994. Circulatory anatomy in bimodally breathing fish. *American Zoology* **34**:280-288.
- Oozeki, Y. and R. Hirano. 1994. Changes in oxygen consumption rate during development of larval Japanese whiting, *Sillago japonica*. *Japanese Journal of Ichthyogy* **41**: 207-214.
- Palzenberger, M. and H. Pohla. 1992. Gill surface area of water-breathing freshwater fish. *Reviews in Fisheries Biology* **2**:187-216.
- Parenti, L.R. and M. Rauchenberger. 1989. Systematic Overview of the Poeciliines. Pages 3- 12 in G.K. Meffe and F.F. Snelson, Jr., editors. *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, New Jersey. 453 p.
- Perez, J.E. and M.K. Rylander. 1985. Hemoglobin heterogeneity in Venezuelan fishes. *Comparative Biochemistry and Physiology B* **80**:641-646.
- Perry, S.F. and K.M. Gilmore. 1996. Consequences of catecholamine release on ventilation and blood oxygen transport during hypoxia and hypercapnia in an elasmobranch (*Squalus acanthias*) and a teleost (*Oncorhynchus mykiss*). *Journal of Experimental Biology* **199**:2105-2118.
- Perry S.F. and P. Laurent. 1993. Environmental Effects on Fish Gill Structure and Function. Pages 231-263 in J. C. Rankin and F. B. Jensen editors. *Fish Ecophysiology*. Chapman and Hall, London. 421 p.
- Perry S.F. and G. McDonald. 1993. Gas Exchange. Pages 251-278 in D.H. Evans editor. *The Physiology of Fishes*. Marine Science Series. CRC Press, Boca Raton, FL. 592 p.

- Perry S.F. and C.M. Wood. 1989. Control and coordination of gas transfer in fishes. *Canadian Journal of Zoology* **67**:2961-2970.
- Petersen, J.K. and G.I. Petersen. 1990. Tolerance, behavior and oxygen consumption in the sand goby, *Pomatoschistus minutus* (Pallas), exposed to hypoxia. *Journal of Fish Biology* **37**:921-933.
- Peterson, M.S. 1990. Hypoxia-induced physiological changes in two mangrove swamp fishes: Sheepshead minnow, *Cyprinodon variegatus* Lacepede and Sailfin molly, *Poecilia latipinna* (Lesueur). *Comparative Biochemistry and Physiology A* **97**:17-21.
- Pichavant, K., J. Person Le Ruyet, N. Le Bayon, A. Severe, A. Le Roux, L. Quemener, V. Maxime, G. Nonnette, and G. Boefe. 2000. Effects of hypoxia on growth and metabolism of juvenile turbot. *Aquaculture* **188**:103-114.
- Post, J.R. and J.A. Lee. 1996. Metabolic ontogeny of teleost fishes. *Canadian Journal of Fisheries and Aquatic Sciences* **53**:910-923.
- Potvin, C. & M. J. Lechowicz. 1990. The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures. *Ecology* **71**:1389-1400.
- Ptacek, M.B. and J. Travis. 1998. Inter-population variation in male mating behaviors in the sailfin molly, *Poecilia latipinna*. *Animal Behaviour* **52**:59-71.
- Quinn, T. and D.E. Schneider. 1991. Respiration of the teleost fish *Ammodytes hexapterus* in relation to its burrowing behavior. *Comparative Biochemistry and Physiology A* **98**:71-75.
- Rabalais, N.N., R.E. Turner, W.J. Wiseman, and Q Dortch. 1998. Consequences of the 1993 Mississippi river flood in the Gulf of Mexico. *Regulated Rivers-Research & Management*. **14**:161-177.
- Rai, H. and G. Hill. 1981. Physical and chemical studies of Lago Tupe; a Central Amazonian blackwater "Ria Lake." *Internationale Revue der gesamten Hydrobiologie*. **66**:37-82.
- Randall, D. 1990. Control and Co-ordination of Gas Exchange in Water Breathers. Pages 253-277 in R.G. Boutilier, editor. *Advances in Comparative and Environmental Physiology*. Vol. VI. Springer-Verlag Press, Berlin. 411 p.
- Rantin, F.T. and K. Johansen. 1984. Responses of the teleost *Hoplias malabaricus* to hypoxia. *Environmental Biology of Fishes* **11**:221-228.

- Rantin, F.T., A.L. Kalinin, M.L. Glass, and M.N. Fernandes. 1992. Respiratory responses to hypoxia in relation to mode of life in two erythrinid species (*Hoplias malabaricus* and *Hoplias lacerdae*). *Journal of Fish Biology* **41**:805-812.
- Reznick, D.N. and H. Bryga. 1987. Life history evolution in guppies (*Poecilia reticulata*): I. Phenotypic and genetic changes in an introduction experiment. *Evolution* **41**:1370-1385.
- Ricker, W.E. 1984. Computation and uses of central trend lines. *Canadian Journal of Zoology* **62**:1987-1905.
- Riggs, A. 1970. Properties of Fish Hemoglobins. Pages 209-252 in W.S. Hoar and D.J. Randall, editors. *Fish Physiology*. Vol IV. The Nervous System, Circulation, and Respiration. Academic Press, New York.
- Riggs, A., H.J. Fyhn, U.E.H. Fyhn, and R.W. Noble. 1979. Studies of functional properties of hemoglobin of *Hoplias malabaricus* and *Hoplerethrinus unitaeniatus*. *Comparative Biochemistry and Physiology A* **62**:189-193.
- Roberts, T.R. 1975. Geographical distribution of African freshwater fishes. *Zoological Journal of the Linnean Society* **57**:249-319.
- Rosenberger A.E. and L.J. Chapman. 2000. Respiratory characters of three species of haplochromine cichlids: Implications for use of wetland refugia. *Journal of Fish Biology* **57**:483-501.
- Rosenberg, R. 1985. Eutrophication, the future marine coastal nuisance? *Marine Pollution Bulletin* **16**:227-231.
- Rosenberg, R; L.O. Loo, and P. Moeller. 1992. Hypoxia, salinity and temperature as structuring factors for marine benthic communities in a eutrophic area. *Netherlands Journal of Sea Research* **30**:121-129
- Saint-Paul, U. and G.M. Soares. 1987. Diurnal distribution and behavioral responses of fishes to extreme hypoxia in an Amazon floodplain lake. *Environmental Biology of Fishes* **20**:91-104.
- Schofield, P.J. and L.J. Chapman. 2000. Hypoxia tolerance of introduced Nile perch: implications for survival of indigenous fishes in the Lake Victoria basin. *African Zoology* **35**:35-42.
- Scott, A.L. and W.A. Rogers. 1981. Haematological effects of prolonged sublethal hypoxia on channel catfish *Ictalurus punctatus* (Rafineque). *Journal of Fish Biology* **18**:591-601.

- Scrimshaw, N.S. 1945. Embryonic development in poeciliid fishes. *Biological Bulletin* **88**:233-246.
- Secor, D.H. and T.E. Gunderson. 1998. Effects of hypoxia and temperature on survival, growth and respiration of juvenile Atlantic sturgeon, *Acipenser oxyrinchus*. *Fishery Bulletin* **96**:603-613.
- Sediva, A., V. Kovac, and G.C. Copp. 2000. Growth variability of morphometric character in perch (*Perca fluviatilis*) and its relation to microhabitat use. *Folia Zoology* **49**:123-132.
- Seliger, H.H., J.A. Boggs, and W.H. Biggley. 1985. Catastrophic anoxia in the Chesapeake Bay in 1984. *Science* **228**:70-73.
- Seki, H., T. Tsuji, and A. Hattori. 1974. Effects of zooplankton grazing on the formation of the anoxic layer in Tokyo Bay. *Estuarine Coastal Marine Science* **2**:145-151.
- Sheinbaum, S. 1979. Reproductive cycles, sex ratios, brood sizes, and adult sizes of estuarine and freshwater populations of the sailfin molly, *Poecilia latipinna* (LeSueur), in the Tampa Bay area. M.S. Thesis, University of South Florida, Tampa, FL. 111 p.
- Simanek, D.E. 1978. Genetic variability and population structure of *Poecilia latipinna*. *Nature* **276**:612-614.
- Simon, J.L. 1974. Tampa Bay estuarine system- a synopsis. *Florida Scientist* **37**:205-216.
- Simpson, D.G. and G. Gunter. 1956. Notes on habitats, systematic characters and life histories of Texas salt water Cyprinodonts. *Tulane Studies in Zoology* **4**:115-134.
- Smith, F.M. and D.R. Jones. 1982. The effect of changes in blood-oxygen carrying capacity on ventilation volume in the rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology* **97**:325-334.
- Smith, M.J. and A.G. Heath. 1978. Use of a modified anaerobic pathway by mirrow carp as an adaptation to hypoxia. *American Zoology* **18**:655.
- Smith, R.B. 1986. The effect of food on reproduction in the sailfin molly (*Poecilia latipinna* (Poeciliidae)). M.S. Thesis, University of Central Florida, Orlando, FL. 97 p.

- Smith, R.E. 1988. Variation in female somatic condition and its relationship to reproduction in the sailfin molly, *Poecilia latipinna* (Pisces: Poeciliidae). M.S. Thesis, University of Central Florida, Orlando, FL. 89 p.
- Snelson, F.F., Jr. 1982. Indeterminate growth in males of the sailfin molly, *Poecilia latipinna*. *Copeia* **1982**:296-302.
- Snelson, F.F., Jr. 1984. Seasonal maturation and growth of males in a natural population of *Poecilia latipinna*. *Copeia* **1984**:252-255.
- Snelson, F.F., Jr. 1985. Size and morphological variation in males of the sailfin molly, *Poecilia latipinna*. *Environmental Biology of Fishes* **13**:35-47.
- Snelson, F.F., Jr., J.D. Wetherington. 1980. Sex ratio in the sailfin molly, *Poecilia latipinna*. *Evolution* **34**:308-319.
- Snelson, F.F., Jr., J.D. Wetherington, and H.L. Large. 1986. The relationship between interbrood interval and yolk loading in a generalized poeciliid fish, *Poecilia latipinna*. *Copeia* **1986**:295-304.
- Soivio, A., M. Nikinmaa, and K. Westman. 1980. The blood oxygen binding properties of hypoxic *Salmo gairdneri*. *Comparative Physiology* **136**:83-87.
- Soivio, A. and H. Tuurula. 1981. Structural and circulatory responses to hypoxia in secondary lamellae of *Salmo gairdneri* gills at two temperatures. *Journal of Comparative Physiology* **145**:37-43.
- Subrahmanyam, C.B. and S.H. Drake. 1975. Studies of the animal communities in two North Florida salt marshes. *Bulletin of Marine Science* **25**:445-465.
- Tetens, V. and N.J. Christensen. 1987. Beta- adrenergic control of blood oxygen affinity in acutely hypoxia-exposed rainbow trout. *Journal of Comparative Physiology B* **157**:667-675.
- Tetens, V. and G. Lykkeboe. 1981. Blood respiratory properties of rainbow trout, *Salmo gairdneri*: responses to hypoxia acclimation and anoxic incubation of blood in vitro. *Journal of Comparative Physiology* **145**:117-125.
- Thibault, R.E. and R.J. Schultz. 1978. Reproductive adaptations among viviparous fishes (Cyprinodontiformes: Poeciliidae). *Evolution* **32**:320-333.
- Thomas, S. and G.M. Hughes. 1982. Effects of hypoxia in blood gas and acid-base parameters of sea bass. *Journal of Applied Physiology* **53**:1336-1341.

- Thiessen, D.D. and S.K. Sturdivant. 1977. Female pheromone in the black molly fish (*Molliensia latipinna*): a possible metabolic correlate. *Journal of Chemical Ecology* **3**:207-217.
- Travis, J. and J.C. Trexler. 1987. Regional variation in habitat requirements of the sailfin molly, with special reference to the Florida Keys, Nongame Wildlife Technical Report No. 3. Florida Game and Freshwater Fish Commission, Tallahassee, FL.
- Travis, J. and B.D. Woodward. 1989. Social context and courtship flexibility in male sailfin mollies, *Poecilia latipinna* (Pisces, Poeciliidae). *Animal Behavior* **38**:1001-1011.
- Travis, J., J.A. Farr, M. McManus, and J.C. Trexler. 1989. Environmental effects on adult growth in the male sailfin molly (*Poecilia latipinna*, Poeciliidae). *Environmental Biology of Fishes* **26**:119-127.
- Trexler, J.C. 1985. Variation in degree of viviparity in the sailfin molly, *Poecilia latipinna*. *Copeia* **1985**:999-1004.
- Trexler, J.C. 1986. Geographic variation in the sailfin molly, *Poecilia latipinna* (Pisces:Poeciliidae):I. Field experiment. *Evolution* **44**:143-156.
- Trexler, J.C. 1988. Hierarchical organization of genetic variation in the sailfin molly, *Poecilia latipinna* (Pisces, Poeciliidae). *Evolution* **42**:1006-1017.
- Trexler, J.C. 1997. Resource availability and plasticity in offspring provisioning: embryo nourishment in sailfin mollies. *Ecology* **78**:1370-1381.
- Trexler, J.C., R.C. Tempe, and J.Travis. 1994. Size-selective predation of sailfin mollies by two species of heron. *OIKOS* **69**:250-258.
- Trexler, J.C. and J. Travis. 1990. Phenotypic plasticity in the sailfin molly, *Poecilia latipinna* (Pisces: Poeciliidae). I. Field experiments. *Evolution* **44**:143-156.
- Truchot, J.P. and A. Duhamel-Jouve. 1980. Oxygen and carbon dioxide in the marine intertidal environment: diurnal and tidal changes in rockpools. *Respiratory Physiology* **39**:241-254.
- Tun, N. and A.H. Houston. 1986. Temperature, oxygen, photoperiod, and the hemoglobin system of the rainbow trout, *Salmo gairdneri*. *Canadian Journal of Zoology* **64**:1883-1888.

- Tuurula, H., P. Part, M. Nikinmaa, and A. Soivio. 1984. The basal channels of secondary lamellae in *Salmo gairdneri* gills – a non-respiratory shunt. *Comparative Biochemistry and Physiology A* **79**:35-39.
- Tyler, M.A. 1984. Dye tracing of a subsurface chlorophyll maximum of a red-tide dinoflagellate to surface frontal regions. *Marine Biology* **78**:285-300.
- Ultsch, G.R., H. Boschung, and M.J. Ross. 1978. Metabolism, critical oxygen tension and habitat selection in darters (*Etheostoma*). *Ecology* **59**:99-107.
- Val, A.L., E.G. Affonso, and V.M. de Almeida-Val. 1992. Adaptive features of Amazon fishes: blood characteristics of Curimata (*Prochilodus* cf. *nigricans*, Osteichthyes). *Physiological Zoology* **65**:832-843.
- Val, A.L. and V.M.F. de Almeida-Val. 1995. Gas Exchange. Pages 70-136 in S.D. Bradshaw, W. Burggren, H.C. Heller, S. Ishii, H. Langer, G. Neuweiler, D.J. Randall, editors. *Fishes of the Amazon and their Environment: Physiological and Biochemical Aspect. Zoophysiology Volume 32.* Springer- Verlag, Berlin. 224 p.
- Val, A.L., V.M. de Almeida-Val, and E.G. Affonso. 1990. Adaptive features of Amazon fishes: Hemoglobins, hematology, intraerythrocytic phosphates and whole blood Bohr effect of *Pterygoplichthys multiradiatus* (Siluriformes). *Comparative Biochemistry and Physiology B* **97**:435-440.
- Van Den Thillart, G. 1982. Adaptation of fish energy metabolism to hypoxia and anoxia. *Molecular Biology* **2**:49-61.
- Verheyen, E., R. Blust, and W. Decler. 1994. Metabolic rate, hypoxia tolerance and aquatic surface respiration of some lacustrine and riverine African cichlid fishes (Pisces: Cichlidae). *Comparative Biochemistry and Physiology A* **107**:403-411.
- Walsh, W.A., C. Swanson, C.S. Lee, J.E. Banno, and H. Eda. 1989. Oxygen consumption by eggs and larvae of striped mullet, *Mugil cephalus*, in relation to development, salinity and temperature. *Journal of Fish Biology* **35**:347-358.
- Webb, P.W. and J.R. Brett. 1972. Oxygen consumption of embryos and parents, and oxygen transfer characteristics within the ovary of two species of viviparous seaperch, *Rhacochilus vacca* and *Embiotoca lateralis*. *Journal of the Fisheries Research Board Canada* **29**:1543-1553.
- Weber, J.M. and D.L. Kramer. 1983. Effects of hypoxia and surface access on growth, mortality, and behavior of juvenile guppies *Poecilia reticulata*. *Canadian Journal of Fisheries and Aquatic Sciences* **40**:1583-1588.


- Weber, R.E. and F.B. Jensen. 1988. Functional adaptations in hemoglobins from ectothermic vertebrates. *Annual Reviews in Physiology* **50**:161-179.
- Weber, R.E. and G. Lykkeboe. 1978. Respiratory adaptations in carp blood influences of hypoxia, red cell organic phosphates, divalent cations and CO₂ on hemoglobin affinity. *Journal of Comparative Physiology* **128**:127-137.
- Weber, R.E., G. Lykkeboe, and K. Johansen. 1976a. Physiological properties of eel haemoglobin: hypoxic acclimation, phosphate effects, and multiplicity. *Journal of Experimental Biology* **64**:75-88.
- Weber, R.E., G. Lykkeboe, and K. Johansen. 1976b. Temperature acclimation and oxygen binding properties of blood and multiple haemoglobins of rainbow trout. *Journal of Experimental Biology* **65**:333-345.
- Weider, L.J. and W. Lampert. 1985. Differential response of *Daphnia* genotypes to oxygen stress: respiration rates, hemoglobin content and low-oxygen tolerance. *Oecologia* **65**:487-491.
- Welcomme, R.L. 1979. *Fisheries Ecology of Floodplain Rivers*. Longman, New York. 317 p.
- Wells, R.M.G., M.D. Ashby, S.J. Duncan, and J.A. MacDonald. 1980. Comparative study of the erythrocytes and haemoglobins in nototheniid fishes from Antarctica. *Journal of Fish Biology* **17**:517-527.
- Wells, R.M.G., R.H. McIntyre, A.K. Morgan, and P.S. Davie. 1986. Physiological stress responses in big gamefish after capture: observations on plasma chemistry and blood factors. *Comparative Biochemistry and Physiology A* **84**:565-571.
- Wells, R.M.G., G.C. Grigg, L.A. Beard, and G. Summers. 1989. Hypoxic responses in a fish from a stable environment: blood oxygen transport in the antarctic fish *Pagothenia borchgrevinki*. *Journal of Experimental Biology* **141**:97-111.
- Wells, R.M.G. and J. Baldwin. 1990. Oxygen transport potential in tropical reef fish with special reference to blood viscosity and hematocrit. *Journal of Experimental Marine Biology and Ecology* **141**:131-143.
- Wells, R.M.G. and R.E. Weber. 1991. Is there an optimum hematocrit for rainbow trout, *Oncorhynchus mykiss* (Walbaum)? An interpretation of recent data based on blood viscosity measurements. *Journal of Fish Biology* **38**:53-65.

- Wetherington, J.D., R.A. Schenck, and R.C. Vrijenhoek. 1989. The origins and ecological success of unisexual *Poeciliopsis*: the frozen niche-variation model. Pages 259-275 in G.K. Meffe and F.F. Snelson, Jr., editors. Ecology and evolution of livebearing fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, New Jersey. 453 p.
- Wetzel, R.G. 1975. Limnology. Saunders, Philadelphia. 743 p.
- Wimberger, P.H. 1991. Plasticity of jaw and skull morphology in the neotropical cichlids *Geophagus brasiliensis* and *G. steinachneri*. *Evolution* **45**:1545-1561.
- Wimberger, P.H. 1992. Plasticity of fish body shape. The effects of diet, development, family and age in two species of *Geophagus* (Pisces; Cichlidae). *Biological Journal of the Linnean Society* **45**:197-218.
- Winberg, G.G. 1956. Rate of metabolism and food requirements in fishes. Translation series No. 194. Fisheries Research Board of Canada Biological Station. Nanaimo, British Columbia.
- Winberg, G.G. 1961. New information on metabolic rate in fishes. Transactions Series No. 362. Fisheries Research Board Canada. Nanaimo, British Columbia (translated from Russian).
- Wourms, J.P. 1981. Viviparity: the maternal:fetal relationship in fishes. *American Zoology* **21**:473-515.
- Wootton, R.J. 1990. Ecology of Teleost Fishes. Chapman and Hall, New York. 404 p.
- Yamamoto, K.I., Y. Itazawa and H. Kobayashi. 1985. Direct observation of fish spleen by an abdominal window method and its application to exercised and hypoxic yellowtail. *Japanese Journal of Ichthyology* **31**:427-433.
- Yeager, D.P. and G.R. Ultsch. 1989. Physiological regulation and conformation: a BASIC program for the determination of critical points. *Physiological Zoology* **62**: 888-907.

BIOGRAPHICAL SKETCH

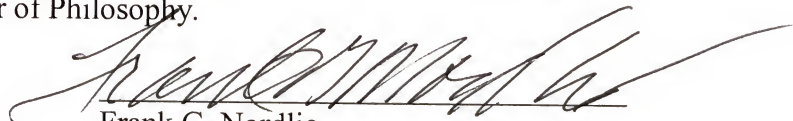
Cindy M. Timmerman was born in Sacramento, California, on September 11, 1968. The youngest of two children in a military family, she grew up traveling. As a child, she lived in North Carolina, Guam, Louisiana, Nebraska, Germany, and Arkansas. She earned her Bachelor of Science and master's degree at the University of Arkansas, Fayetteville, where she met her husband, Rudy Timmerman. Shortly thereafter, they moved to Gainesville, Florida, so she could pursue her doctorate in zoology. Upon completion of the experimental work required for her dissertation, she returned to Arkansas in 1998. She currently lives with her husband, daughter, three dogs, and two cats in the boon docks of the Ozark Mountains.

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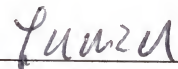
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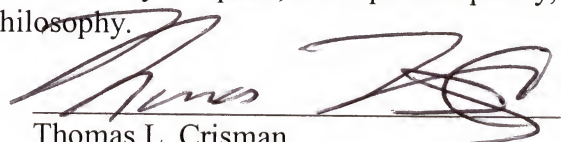
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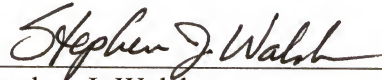
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May 2001

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